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(54) Title: RECEPTORS FOR BOMBESIN-LIKE PEPTIDES

(57) Abstract

Receptors for bombesin-like peptides are solubilized and purified in active form. The amino acid sequence and DNA encoding various subtypes of the receptors are disclosed. Uses of the purified receptor gene and polypeptide are disclosed, including means for screening for agonists and antagonists of the receptor ligands, for producing diagnostic or therapeutic reagents, and for producing antibodies. Therapeutic or diagnostic reagents and kits are also provided.

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RECEPTORS FOR BOMBESIN-LIKE PEPTIDES

This application is a continuation-in-part of U.S. Patent Application Serial No. 07/670,603 filed on March 15, 1991; of U.S. Patent Application Serial No. 07/533,659 filed on June 5, 1990; and of U.S. Patent Application Serial No. 07/426,150 filed on October 24, 1989; each of which is incorporated herein by reference and benefit is claimed of the respective filing dates.

Field of the Invention

The present invention relates generally to nucleic acids and polypeptides characteristic of receptors for bombesin-like peptides, and more particularly to their uses in preparing new reagents useful for diagnosing or treating various receptor related medical conditions.

BACKGROUND OF THE INVENTION

Growth factors are involved in numerous physiological and pathological processes. An increasing number of small regulatory peptides have been discovered in the neural and neuroendocrine cells of mammalian tissues. More recent evidence has pointed to the role of neuropeptides in the regulation of animal cell growth, e.g., in the action of mitogenic peptides on the Swiss 3T3 cell system. One of the first neuropeptides studied was the tetra-decapeptide bombesin which was originally isolated from amphibian skin, Anastasi et al. (1971) Experientia 27:166-167. Over ten bombesin-related peptides have been subsequently isolated from various sources and classified into three subfamilies according to their C-terminal sequences. These subfamilies are the bombesin, the ranatensin, and litorin subfamilies.

Several endogenous mammalian peptides are structurally related to bombesin-like peptides. The gastrin releasing peptide (GRP) is a member of the bombesin subfamily, and neuromedin B (NMB) is a member of the ranatensin subfamily.

Gastrin releasing peptide (GRP) is a 27 amino acid peptide having the following sequence in humans:
NH_2-Val-Pro-Leu-Pro-Ala-Gly-Gly-Gly-Thr-Val-Leu-Thr-Lys-Met-Tyr

-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-(NH_2). GRP is of significant interest because it functions as an autocrine growth factor in the pathogenesis of cancer. In particular, GRP has been found to promote the growth of human small cell lung carcinoma (SCLC). GRP binding to cell surface receptors is thought to stimulate cellular growth by promoting the hydrolysis of phosphatidyl inositides and by activating protein kinase C. A large number of biological responses to GRP have been observed including: stimulation of Na^+/H^+ antiport, mobilization of intracellular Ca^{2+}, transient expression of c-fos and c-myc proto-oncogenes, induction of tyrosine kinase activity, elevation of DNA synthesis, and promotion of cell division.

Other bombesin-like peptides, including neuromedin B, mediate a variety of similar biological and pharmacological activities. These peptides appear to function as growth factors, and to be involved in regulation of homeostasis, thermoregulation, metabolism, and behavior.

For example, the role of GRP in maintaining the growth of SCLC suggests that effective therapeutic agents could be developed to interrupt the autocrine growth cycle by inactivating GRP or inhibiting its receptor. The active site of GRP is the C-terminal region which binds high affinity receptors on SCLC membranes. Blocking this binding can inhibit SCLC growth. This has already been accomplished with monoclonal antibodies to bombesin which bind to the active site on GRP, thus inactivating the peptide, see Cuttitta et al. (1985) Nature 316:823-826.

Another means to block GRP from binding to its receptor, and therefore useful in treating SCLC, is to inhibit the receptor itself. Unfortunately, means to find such reagents have been severely hampered by the absence of purified GRP receptor in an active form. This problem can be overcome by use of the recombinant receptor. Along with providing an improved renewable source of the receptor from a specific source, using the recombinant GRP receptor in screening for GRP receptor reactive drugs also has the following advantages: a

potentially greater number of receptors per cell giving greater yield of reagent and higher signal to noise ratio in assays; and receptor subtype specificity (giving greater biological and disease specificity).

Cross-linking of the GRP receptor to bound radiolabeled GRP has been used to visualize the GRP receptor-ligand conjugate on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and to deduce certain other characteristics of the receptor, see Rozengurt et al., PCT/GB88/00255. However, the technique used did not involve isolation of the receptor, but rather involved characterization of a modified form of the receptor protein. Unfortunately, in order to characterize the structural properties of the GRP receptor in greater detail and to understand the mechanism of action at the molecular level, the receptor needs to be purified. For many applications, the receptor must be isolated in an active state retaining the binding activity of the receptor. These applications include the generation of antibodies against active receptor epitopes, structural studies of the ligand binding site, and the use of the purified receptor for screens for agonists and antagonists of GRP binding. Isolation of the receptor gene should provide an economical source of the receptor, allow expression of more receptors on a cell leading to increased assay sensitivity, promote characterization of various receptor subtypes, and allow correlation of activity with receptor structures.

Similarities in other bombesin-like peptide functions exist. In particular, the NMB receptor shares many functions and characteristics with the GRP receptor, but also exhibits different structural and functional properties. To date, few receptors have been isolated and characterized in their active form. The amount of receptor present in most tissues is minute. Furthermore, the receptor must often be solubilized from membranes with detergents that can perturb or disrupt the structure of the receptor protein. Further compounding these difficulties is the unpredictable nature of receptor isolation in that the method for successfully solubilizing one protein

receptor type or subclass may not be successful for a different protein receptor type or subclass.

Thus, a need exists for the isolation and characterization of receptors for bombesin-like peptides, e.g., GRP, NMB, and other bombesin-like peptides. The present invention provides these and the means for preparing many other useful reagents.

SUMMARY OF THE INVENTION

The present invention provides gene and protein sequences of various receptors for bombesin-like peptides (RBP), including subtypes R1BP and R2BP, which are receptors for GRP and NMB, respectively, as well as other similar receptor molecules, e.g., R3BP.

This invention provides recombinant nucleic acids, and isolated or substantially pure nucleic acids, which are substantially homologous to a sequence encoding a receptor for a bombesin-like peptide, or a fragment thereof. Nucleic acids encoding fusion polypeptides are contemplated, as are vectors, cells, and organisms comprising such nucleic acids. Exemplary embodiments are different RBP subtypes, i.e., R1BP (GRP receptor), R2BP (NMB receptor), and R3BP (a third related gene for a receptor-like protein whose ligand has not yet been identified).

Recombinant polypeptides, and isolated or substantially pure polypeptides derived from these RBP protein sequences are encompassed herein. Fusion polypeptides are provided, along with cells and organisms comprising the polypeptides. Compositions comprising these polypeptides are embraced herein. Exemplary embodiments are, again, GRP receptor, NMB receptor, and R3BP.

The invention provides antibodies specific for epitopes unique to, or characteristic of, the receptors for bombesin-like peptides. These include antibodies which bind specifically to either epitopes which are shared by the genus of receptors for bombesin-like peptides, or epitopes which distinguish between the different receptor subtypes.

Kits comprising any of these compositions are included herein. Thus, various nucleic acid molecules, polypeptides, and antibodies may provide the basis of various diagnostic or therapeutic kits.

The various compositions also provide bases for methods for treating hosts, particularly those suffering from abnormal receptor function, e.g., proliferative cell

conditions, by administering effective amounts of these reagents, or contacting biological samples with them.

The compositions, e.g., ligand binding fragments, also provide the means to select and screen for additional agonists and antagonists for the respective receptor subtypes. Selected compounds are made available, both ligands and molecules which interact at polypeptide regions separate from the ligand binding regions. Of particular utility are compounds affecting multiple receptor subtypes, e.g., those exhibiting desired spectra of specificity for modulating biological activity.

The group of RBP subtypes is also very useful in providing a group of receptor polypeptides having both substantial similarities and critical differences. These RBP, as a group, allow dissecting of structure and function for the class in a manner impossible from characterization of a single subtype.

The following description specifically describes mostly the mouse R1BP (GRP receptor), but similar concepts could be applied to other related receptors for bombesin-like peptides, including human R1BP (GRP receptor), rat R2BP (NMB receptor), human R2BP (NMB receptor), and human R3BP (incompletely characterized homologous putative receptor). In particular, analogous uses and reagents derived from other similar receptors for bombesin-like peptides will be developed. Identification of new bioactive ligands for new receptor subtypes will also result.

This invention pertains to expressing DNA encoding the GRP receptor in host cells, e.g., transcribing and translating, thereby enabling the synthesis of GRP receptor compositions having the amino acid sequence of the naturally-occurring GRP receptor which are entirely free of other proteins of the species of origin and further enabling the synthesis of novel mutant GRP receptors.

In addition, this invention relates to the use of DNA encoding the GRP receptor or its fragments in hybridization diagnosis of defective GRP receptor DNA or mRNA, and for

obtaining DNA encoding the GRP receptor from natural sources. Similar uses of genes for other receptors for bombesin-like peptides (RBP) are likewise provided, e.g., R2BP (NMB receptor subtype), R3BP, or other closely related receptors.

More specifically, this invention pertains to the use of the recombinant R1BP, R2BP, or R3BP, and related proteins; to cell lines transfected with vectors directing the expression of R1BP, R2BP, R3BP, or related receptors; to membranes from such cell lines, e.g., in drug screening assays for compounds having suitable binding affinity for the respective receptors, individually or in combination; and to antibodies and other reagents made available therefrom.

Even more specifically, this invention pertains to recombinant R1BP (GRP-R), R2BP (NMB-R), or R3BP, along with protein fragments of the receptors, and antibodies directed thereto, that are useful in diagnostic assays to determine the levels of expression in a patient's tissues of the respective receptor subtypes. Assays based on detection of antibodies to the receptors and/or detection of the receptors can also have prognostic value.

Additionally, this invention pertains to using the recombinant receptors or fragments or derivatives thereof, e.g., to make reagents such as antibodies to the receptors or fragments, or to isolate specific receptor agonists or antagonists defined in screening assays.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphic comparison of the ability of several detergents to solubilize R1BP (GRP receptor) and shows the effect of solubilization on binding activity.

Figure 2 is a graph of GRP-binding activity and R1BP (GRP receptor) solubilization as a function of detergent (CHAPS) concentration.

Figure 3 is a graph of R1BP solubilization and activity as a function of the soluble cholesteryl ester stabilizing agent (CHS) concentration.

Figure 4 is a graph of GRP binding activity as a function of detergent (CHAPS) concentration.

Figure 5 is a gel display of SDS-PAGE analysis of ^{125}I-GRP cross-linked to R1BP (GRP-R) in a crude soluble extract.

Figure 6 is a silver stained gel display of SDS-PAGE analysis of the purified R1BP.

Figure 7 shows the separation of tryptic fragments of R1BP by reverse-phase HPLC.

Figure 8 shows a hydropathy analysis of the deduced amino acid sequence of the mouse R1BP (GRP-R). This was generated using the Pepplot (window = 20 amino acids) in the Sequence Analysis Software Package of the University of Wisconsin Genetics Group, see Devereaux et al (1984) Nuc. Acids Res. 12:387-395, which is incorporated herein by reference. Positive regions are relatively hydrophobic, and negative regions are hydrophilic. Putative transmembrane domains are numbered sequentially by numbers I through VII. Solid line: Kyte-Doolittle criterion, Dotted line: Goldman criterion.

Figure 9 shows Northern hybridization analysis of mRNA from Swiss 3T3 cells.

Figure 10 shows Northern hybridization analysis of mRNA from human fetal lung cells (HFL).

Figure 11 shows GRP ligand-dependent induction of chloride current in a Xenopus oocyte expressing an in vitro transcript from a R1BP (GRP-R) cDNA clone.

Figure 12 shows a hydropathy analysis plot of the deduced amino acid sequence of a rat NMB receptor.

Figure 13 shows a hydropathy plot derived from a human GRP receptor.

Figure 14 shows biological response of receptors for two bombesin-like peptides. This figure shows the increase in intracellular-Ca^{2+} of NCI-H345 cells in response to Tyr^4-bombesin (BN) and neuromedin B (NMB). An increase in fluorescence at 492 nm appears after addition of the indicated ligand. The ligands were added to achieve a final concentration of 100 nM. 10 µl 10% Triton-X was added as indicated at the termination of the experimental determination. 5 X 10^6 cells were used per determination. The time scale is displayed at the lower right hand corner of the figure.

Figure 15 shows a concentration effect relationship of NMB or BN in NCI-H345 cells. Data are shown as nM change from resting baseline values. The values are the mean of 2-3 separate determinations. 5 X 10^6 cells were used in each assay. ✕: NMB agonist response, ▲:Bombesin response.

Figure 16 shows concentration effect relationship of the antagonist or inhibitor, [D-Phe^6]BN(6-13) ethyl ester, in the presence of 50 nM NMB or BN in NCI-H345 cells. The inhibitor was incubated with the cells for 5 minutes prior to the addition of ligand. The percent change in $[Ca^{2+}]_i$ was determined as described. The values are the mean of two separate determinations. 5 X 10^6 cells were used per determination. ✕:NMB response, ▲:Bombesin response.

Figure 17 shows a hydropathy plot derived from a human R2BP (NMB-R).

Figure 18 shows functional expression of a human R1BP (GRP-R) and human R2BP (NMB-R). The electrophysiological response (chloride current versus time) is shown of Xenopus oocytes to GRP or NMB application after expression of injected human GRP-receptor mRNA or NMB-receptor transcribed from cDNA clone templates in vitro. Panel A shows GRP-R response to agonists (10 nM) and to [D-Phe^6]BN(6-13) (1 µM), plus agonists (10 nM). Panel B shows NMB-R response to agonists (10 nM) and

to [D-Phe^6]BN(6-13), (1 μM) plus agonists (10 nM). Uninjected oocytes did not respond to GRP or NMB.

Figure 19 shows RNase protection analysis of steady state R1BP (GRP-R) mRNA and R2BP (NMB-R) mRNA levels in various lung cancer cell lines. 30 μg of total RNA was hybridized to either a ^{32}P-labeled GRP-R or NMB-R cRNA probe as described. A portion of a resulting autoradiograph is shown;

A) R1BP (GRP-R), 5 day exposure in the presence of an intensifying screen;

B) R2BP (NMB-R), 12 day exposure in the presence of an intensifying screen.

The results from all cell lines examined are summarized in Table 10. The signal strength on the resulting autoradiograph was assessed and assigned a relative value that is exemplified by the following in Figure 19:

++	NCI-H345	+	NCI-N592
tr	NCI-H510	-	NCI-H209

To ascertain that equivalent amounts of intact RNA was analyzed, total RNA from each sample analyzed was also electrophoresed, blotted, and probed with human beta-actin. Signals from all RNA samples were comparable, indicating that the RNA analyzed is not degraded. RNA from the human glioblastoma cell line U118 was included as a positive control in the GRP-R experiments.

Figure 20 shows results of a low stringency genomic blot of human DNA cut with Eco RI. A mouse R1BP (GRP-R) probe was used, revealing six new fragments, none of which corresponds to the receptors earlier characterized herein. The six novel bands are indicated by the arrows.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

CONTENTS

I. General

The present invention provides the amino acid sequence and DNA sequence of various receptors for bombesin-like peptides, e.g., a mouse receptor subtype one for a bombesin-like peptide, also designated R1BP, which corresponds to a gastrin-releasing peptide (GRP) receptor. These sequences were obtained after an R1BP, or GRP receptor (GRP-R), was purified and the amino acid sequence of tryptic fragments of the receptor was determined. Similar sequences for a human R1BP (GRP-R), a rat receptor subtype two for a bombesin-like peptide, i.e., a neuromedin B receptor (NMB-R), a human NMB-R, and a human third receptor subtype, designated R3BP, are provided. The descriptions below are often directed to a mouse R1BP (GRP-R) but are likewise applicable to other receptor subtypes. Human R1BP, rat R2BP, human R2BP, and human R3BP are exemplary embodiments of the class of RBP.

Partial amino acid sequences obtained from a purified R1BP were used to deduce DNA probes which were then used to isolate an R1BP cDNA form of the gene. Some of the standard methods are described or referenced, e.g., in Maniatis et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols 1-3, CSH Press, NY; Ausubel et al., Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel et al. (1987 and Supplements) Current Protocols in Molecular Biology,

Greene/Wiley, New York; all of which are each incorporated herein by reference. Isolation of this R1BP gene allowed isolation of a gene for a homologous second subtype, R2BP, commonly referred to as a NMB receptor, which further led to the isolation of a third subtype, designated R3BP. These genes will allow isolation of other receptor genes for bombesin-like peptides, further extending the family beyond the herein described three subtypes, and five specific embodiments. The procedure is broadly set forth below.

A cDNA library, constructed in lambda gt10 bacteriophage, was prepared from RNA isolated from Swiss 3T3 cells. Several modifications and unique techniques had to be utilized to overcome problems associated with isolating a cDNA clone when probing the library with oligonucleotides. In particular, it was necessary to enrich the library for cDNA species encoding the R1BP due to the under representation of such species in unenriched cDNA libraries. Oligonucleotide probes were designed having a nucleotide sequence based upon the most likely codon usage. The cDNA library was plated out, allowing the lambda phage containing cDNA inserts to lyse their E. coli hosts and form plaques, each containing individual cDNA inserts. The plaques were screened for R1BP DNA sequences with labeled oligonucleotide probes. Subtype one RBP cDNA species were isolated, but these encoded an incomplete R1BP.

Polymerase chain reaction technology was used to isolate additional cDNA species encoding portions of an R1BP (GRP-R), and its 5' and 3' flanking regions. Gene-specific primer-directed cDNA cloning was then used to obtain a single cDNA clone encoding an entire receptor subtype one translation product. The actual cloning techniques utilized herein are set forth in detail in Examples 12 and 13 below. Using the isolated subtype one receptor gene from mouse, a homologous second subtype (R2BP, or NMB receptor) was isolated from rat. Similarly, human R1BP and R2BP sequences have been isolated, along with a third subtype, designated R3BP, which is as yet incompletely characterized.

Once a cDNA for a receptor subtype one was isolated from mouse, it was sequenced. The nucleotide sequence revealed the amino acid sequence of the primary translation product of a GRP receptor, i.e., the amino acid sequence before any post-translational modification.

A complete mouse amino acid sequence is shown in Table 1. This sequence corresponds to SEQ ID NO: 1. Table 1 discloses both the nucleotide sequence of the receptor subtype one, which binds GRP, and its deduced amino acid sequence, also published in Battey et al. (1991) Proc. Nat'l Acad. Sci. USA 88:395-399, which is incorporated herein by reference. The experimentally determined amino acid sequence of the intact receptor subtype one protein and of isolated tryptic peptides to the receptor are indicated by underlining. Putative transmembrane sequences are labeled I through VII. Consensus sequences for N-linked glycosylation are boxed.

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-377  AAAACTGCAGCCAGAGAGACTCAGTCTAGGATGGAGGTAGGAAGAGCTGAGACAAAGTGGGCTTAATTCTAAGCTTTTCTTCAGGCTGAGTTTCTGTTGC  -278

-277  TTGTTAACTTAGTGAATGTACAGATGTATTGCTTGCTGGTGGTGTGAAGGCTGGGACAGAACCAACATCAACAAACTGAGCTAGAGTTTGGAATACCAGT  -178

-177  GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGAATTCAGAGTGTTTTAAAGAGAGATCAAGAGGCTCACACAGATCAGCGAGCCTAACTGACAAA  -78

-77   CCTTCAGCGCCTAACTGAAAAACCCAGAAGTTACAAAGCAGCATCTTGAAGGCGCATTTGAAGAGAGAAGCTTTGAGATGGCTCCAAATAATTGTTCCCA  22
                                                                                  MetAlaProAsnAsnCysSerHi

 23   CCTGAACTTGGACGTGGACCCTTTCCTGTCCTGCAACGACACCTTCAATCAAAGTCTGAGTCCCCCCAAGATGGACAACTGGTTTCACCCGGGCTTCATC  122
      sLeuAsnLeuAspValAspProPheLeuSerCysAsnAspThrPheAsnGlnSerLeuSerProProLysMetAspAsnTrpPheHisProGlyPheIle

123   TATGTCATCCCTGCAGTTTATGGGCTTATCATCGTGATAGGTCTTATTGGCAACATCACGCTCATCAAGATCTTCTGCACGGTCAAGTCCATGCGAAACG  222
      TyrValIleProAlaValTyrGlyLeuIleIleValIleGlyLeuIleGlyAsnIleThrLeuIleLysIlePheCysThrValLysSerMetArgAsnV

223   TGCCAAACCTGTTCATCTCTAGCCTGGCTTTGGGAGACCTGCTGCTGCTGGTGACATGCGCCCCTGTGGATGCCAGCAAGTACCTGGCTGACAGGTGGCT  322
      alProAsnLeuPheIleSerSerLeuAlaLeuGlyAspLeuLeuLeuValThrCysAlaProValAspAlaSerLysTyrLeuAlaAspArgTrpLe

323   ATTTGGCAGAATTGGCTGCAAACTGATCCCCTTTATACAACTTACTTCAGTGGGGGTGTCTGTCTTCACACTTACGGCACTGTCAGCTGACAGGTACAAA  422
      uPheGlyArgIleGlyCysLysLeuIleProPheIleGlnLeuThrSerValGlyValSerValPheThrLeuThrAlaLeuSerAlaAspArgTyrLys

423   GCCATTGTACGGCCAATGGATATCCAGGCATCCCATGCCCTGATGAAGATCTGTCTCAAAGCTGCTTTGATCTGGATTGTCTCTATGTTGTTGGCCATCC  522
      AlaIleValArgProMetAspIleGlnAlaSerHisAlaLeuMetLysIleCysLeuLysAlaAlaLeuIleTrpIleValSerMetLeuLeuAlaIleP
```

TABLE 1A

TABLE 1B

As used herein, the terms "receptor subtype one for bombesin-like peptides," "R1BP," or "GRP receptor" shall be defined as including a protein or peptide having the amino acid sequence shown in Table 1, or a fragment thereof. It also refers to a polypeptide which functionally and similarly binds to a GRP protein with high affinity, e.g., at least about 100 nM, usually better than about 30 nM, preferably better than about 10 nM, and more preferably at better than about 3 nM. The terms shall also be used herein to refer to a subtype one receptor gene, the alleles of the mouse subtype one receptor, or other subtype one receptors in a mouse, and the subtype one receptors of species other than mouse, for example, humans and other mammals. The term does not encompass natural antibodies which bind the ligand, since the structural features of an antibody binding site are different from ligand binding sites. A human subtype two receptor (R2BP) sequence is shown in Table 2. This sequence corresponds to SEQ ID NO: 3.

Table 2: The nucleotide sequence and predicted amino acid sequence derived from both the human genomic R1BP (GRP-R) clone and from the human SCLC cell line NCI-H345 cDNA. Inverted triangles indicate intron positions as determined from the genomic clone.

SUBSTITUTE SHEET

TABLE 2A

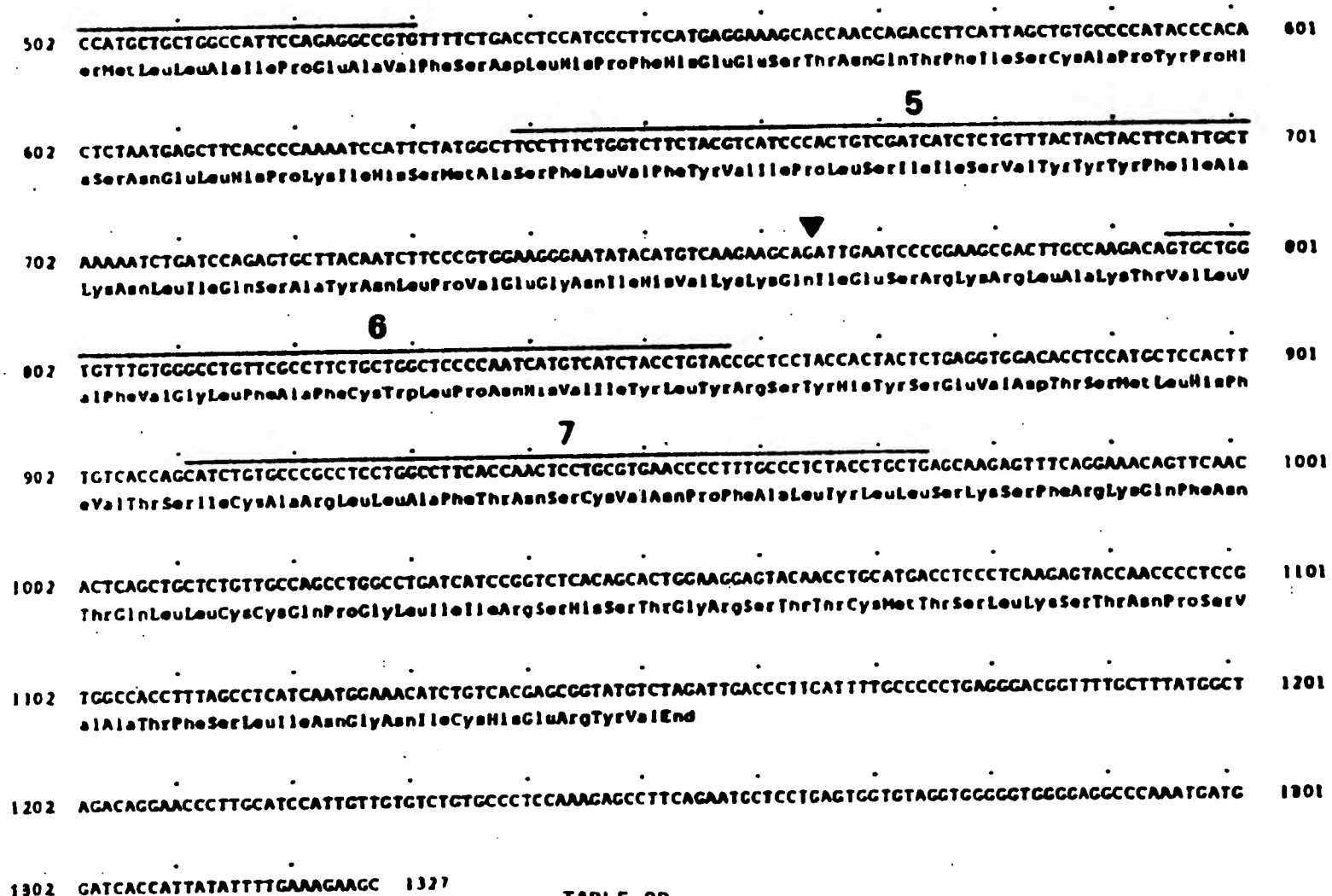

TABLE 2B

SUBSTITUTE SHEET

The mouse subtype one receptor DNA was used as a probe to isolate a rat second subtype receptor gene sequence, see Table 3, and a human subtype one receptor gene sequence, see Table 2. The human subtype one receptor sequence was used as a probe to isolate a human sequence designated R3BP. This designation as a member of the RBP family results from its high homology to receptor subtypes one and two, see Table 12. The subtype one receptor (for bombesin-like peptides) is commonly referred to as a GRP receptor, whereas the subtype two receptor (for bombesin-like peptides) is commonly referred to as a NMB receptor. The sequence in Table 3 corresponds to SEQ ID NO: 5; and the sequence in Table 4 corresponds to SEQ ID NO: 7; and the sequence in Table 12 corresponds to SEQ ID NO: 9. The isolated rat subtype two receptor gene sequence was then used to isolate a human subtype two receptor gene sequence, see Table 4. Similar procedures will be applicable to isolate homologous receptors from other species, or other receptors in the same species, e.g., a human subtype 3 receptor. In particular, receptors for other bombesin-like peptides will be isolated. See Example 29, below, Figure 20, and Table 12.

Table 3: The nucleotide sequence and predicted amino acid sequence derived from two independent rat subtype two receptor (NMB-R) cDNA clones. Horizontal underlining between nucleotide and amino acid sequences indicate the location of seven predicted transmembrane domains (numbered sequentially) based on homology to other G-protein coupled receptor superfamily members. Heavy dots show the location of potential sites of N-linked glycosylation. The sequence is also disclosed in Wada et al. (1991) Neuron 6:421-430, which is incorporated herein by reference.

```
1   GGTGGCTCAGTTCCAGGAGCCACAAACTTGCCAGGATCAGAGACAATCAACTAAACCCAGGTCGTACTCACCGCACTTTCGAGACGCGCGAGTCCAGGAA  100

101 AACTCCCGCGAATCCCCTGGGAAACGACACCATGCCCCCCAGGTCTCTCCCCAACCTCTCCTTGCCCACCGAGGCGAGCGAGAGCGAGTTGGAACCCGAG  200
                                    MetProProArgSerLeuProAsnLeuSerLeuProThrGluAlaSerGluSerGluLeuGluProGlu

201 GTGTGGGAAAATGATTTCCTGCCTGACTCAGACGGGACCACCGCGGAGTTGGTAATCCGCTGTGTGATACCATCCCTCTACCTAATCATCATCTCGGTGG  300
    ValTrpGluAsnAspPheLeuProAspSerAspGlyThrThrAlaGluLeuValIleArgCysValIleProSerLeuTyrLeuIleIleIleSerValG

301 GCTTGCTGCGCAACATCATGCTGGTGAAGATATTCCTCACCAACAGCACCATGCGGAGTGTCCCCAACATCTTCATCTCTAACCTGGCTGCGGGAGACCT  400
    lyLeuLeuGlyAsnIleMetLeuValLysIlePheLeuThrAsnSerThrMetArgSerValProAsnIlePheIleSerAsnLeuAlaAlaGlyAspLe

401 GCTGCTGCTGCTGACCTGCGTCCCAGTGGATGCCTCCCGATACTTCTTTGATGAATGGGTGTTCGGCAAGCTGGGCTGCAAACTCATCCCAGCCATCCAG  500
    uLeuLeuLeuThrCysValProValAspAlaSerArgTyrPhePheAspGluTrpValPheGlyLysLeuGlyCysLysLeuIleProAlaIleGln

501 CTCACCTCGGTGCGGCGTTTCCGTGTTCACTCTCACGGCCCTCAGCGCTGACAGGTACAGAGCTATCGTGAACCCCATGGACATGCAGACGTCTGGTGTGG  600
    LeuThrSerValGlyValSerValPheThrLeuThrAlaLeuSerAlaAspArgTyrArgAlaIleValAsnProMetAspMetGlnThrSerGlyValV

601 TGCTGTGGACCAGTTTGAAGGCCGTGGGCATCTCGGTGGTCTCTGTGCTGTTGGCTGTCCCTGAGGCTGTGTTTTCGGAAGTAGCACGCATCGGTAGCTC  700
    alLeuTrpThrSerLeuLysAlaValGlyIleTrpValValSerValLeuLeuAlaValProGluAlaValPheSerGluValAlaArgIleGlySerSe

701 CGATAACAGCAGTTTCACAGCATGCATACCCTACCCACAAACAGATGAGTTACATCCAAAGATCCACTCAGTGCTCATTTTTCTTGTCTATTTCCTCATA  800
    rAspAsnSerSerPheThrAlaCysIleProTyrProGlnThrAspGluLeuHisProLysIleHisSerValLeuIlePheLeuValTyrPheLeuIle
```

TABLE 3A

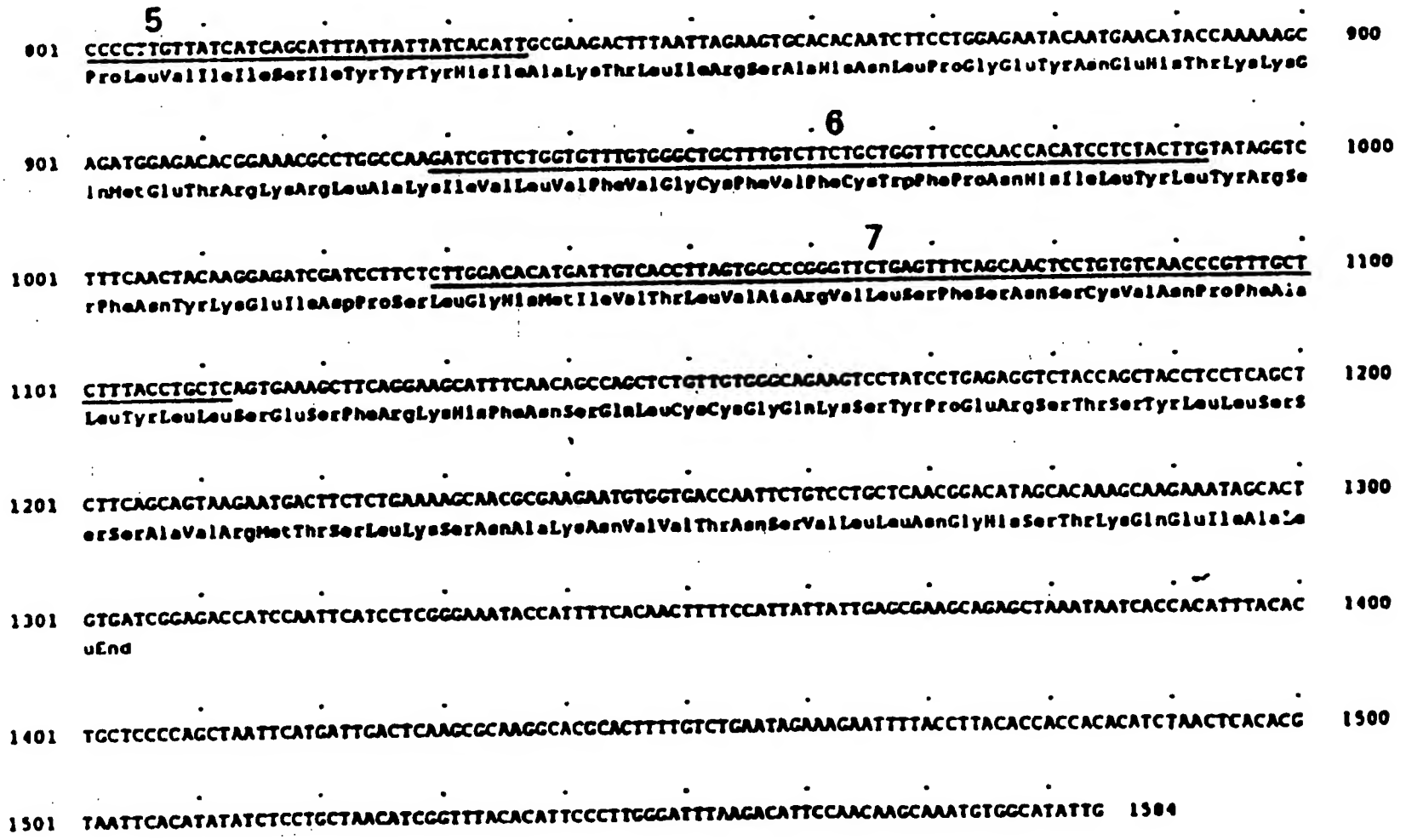

TABLE 3B

SUBSTITUTE SHEET

Table 4: The nucleotide sequence and predicted amino acid sequence derived from human. Both the human genomic receptor subtype one (GRP-R) clone and the human SCLC cell line NCI-H345 cDNA indicate the same protein sequence. Inverted triangles indicate intron positions as determined from the genomic clone.

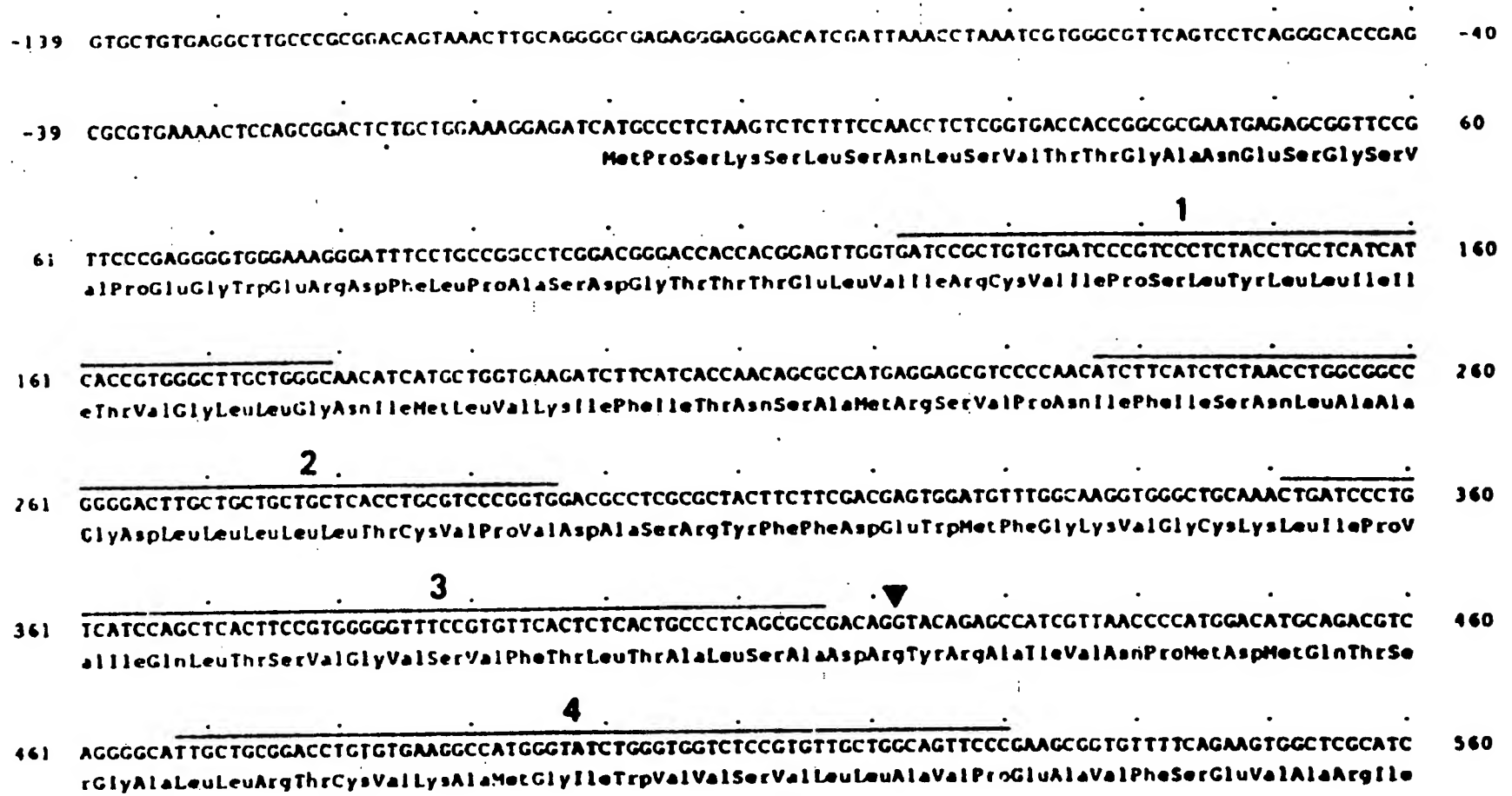
-139 GTGCTGTGAGGCTTGCCCGCGGACAGTAAACTTGCAGGGGCGAGAGGGAGGGACATCGATTAAACCTAAATCGTGGGCGTTCAGTCCTCAGGGCACCGAG -40
-39 CGCGTGAAAACTCCAGCGGACTCTGCTGGAAAGGAGATCATGCCCTCTAAGTCTCTTTCCAACCTCTCGGTGACCACCGGCGCGAATGAGAGCGGTTCCG 60
MetProSerLysSerLeuSerAsnLeuSerValThrThrGlyAlaAsnGluSerGlySerV
1
61 TTCCCGAGGGGTGGGAAAGGGATTTCCTGCCGGCCTCGGACGGGACCACCACGGAGTTGGTGATCCGCTGTGTGATCCCGTCCCTCTACCTGCTCATCAT 160
alProGluGlyTrpGluArgAspPheLeuProAlaSerAspGlyThrThrThrGluLeuValIleArgCysValIleProSerLeuTyrLeuLeuIleIl
161 CACCGTGGGCTTGCTGGGCAACATCATGCTGGTGAAGATCTTCATCACCAACAGCGCCATGAGGAGCGTCCCCAACATCTTCATCTCTAACCTGGCGGCC 260
eThrValGlyLeuLeuGlyAsnIleMetLeuValLysIlePheIleThrAsnSerAlaMetArgSerValProAsnIlePheIleSerAsnLeuAlaAla
2
261 GGGGACTTGCTGCTGCTGCTCACCTGCGTCCCGGTGGACGCCTCGCGCTACTTCTTCGACGAGTGGATGTTTGGCAAGGTGGGCTGCAAACTGATCCCTG 360
GlyAspLeuLeuLeuLeuThrCysValProValAspAlaSerArgTyrPhePheAspGluTrpMetPheGlyLysValGlyCysLysLeuIleProV
3
361 TCATCCAGCTCACTTCCGTGGGGGTTTCCGTGTTCACTCTCACTGCCCTCAGCGCCGACAGGTACAGAGCCATCGTTAACCCCATGGACATGCAGACGTC 460
alIleGlnLeuThrSerValGlyValSerValPheThrLeuThrAlaLeuSerAlaAspArgTyrArgAlaIleValAsnProMetAspMetGlnThrSe
4
461 AGGGGCATTGCTGCGGACCTGTGTGAAGGCCATGGGTATCTGGGTGGTCTCCGTGTTGCTGGCAGTTCCCGAAGCGGTGTTTTCAGAAGTGGCTCGCATC 560
rGlyAlaLeuLeuArgThrCysValLysAlaMetGlyIleTrpValValSerValLeuLeuAlaValProGluAlaValPheSerGluValAlaArgIle

TABLE 4A

```
561  AGTAGCTTGGATAATAGCAGCTTCACAGCATGTATCCCATACCCTCAAACAGATGAATTACATCCAAAGATTCATTCAGTGCTCATTTTCTTGGTCTATT   660
     SerSerLeuAspAsnSerSerPheThrAlaCysIleProTyrProGlnThrAspGluLeuHisProLysIleHisSerValLeuIlePheLeuValTyrP

                                  5
661  TCCTCATACCACTTGCTATTATTAGCATTTATTATTATCATATTGCAAAGACCTTAATTAAAAGCGCCACACAATCTTCCTGGAGAATACAATGAACATAC   760
     heLeuIleProLeuAlaIleIleSerIleTyrTyrTyrHisIleAlaLysThrLeuIleLysSerAlaHisAsnLeuProGlyGluTyrAsnGluHisTh

                                                  6
761  CAAAAAACAGATGGAAACACGGAAACGCCTGGCTAAAATTGTGCTTGTCTTTGTGGGCTGTTTCATCTTCTGTTGGTTTCCAAACCACATCCTTTACATG   860
     rLysLysGlnMetGluThrArgLysArgLeuAlaLysIleValLeuValPheValGlyCysPheIlePheCysTrpPheProAsnHisIleLeuTyrMet

                                                                7
861  TATCGGTCTTTCAACTATAATGAGATTGATCCATCTCTAGGCCACATGATTGTCACCTTAGTTGCCCGGGTTCTCAGTTTTGGCAATTCTTGTGTCAACC   960
     TyrArgSerPheAsnTyrAsnGluIleAspProSerLeuGlyHisMetIleValThrLeuValAlaArgValLeuSerPheGlyAsnSerCysValAsnP

961  CATTTGCTCTTTACCTACTCAGTGAAAGCTTCAGGAGGCATTTCAACAGCCAACTCTGCTGTGGGAGGAAGTCCTATCAAGAGAGAGGAACCAGCTACCT   1060
     roPheAlaLeuTyrLeuLeuSerGluSerPheArgArgHisPheAsnSerGlnLeuCysCysGlyArgLysSerTyrGlnGluArgGlyThrSerTyrLe

1061 ACTCAGCTCTTCAGCGGTCCGTATGACATCTCTGAAAAGCAATGCTAAGAACATGGTGACCAATTCTGTTTTACTAAATGGGCACAGCATGAAGCAGGAA   1160
     uLeuSerSerSerAlaValArgMetThrSerLeuLysSerAsnAlaLysAsnMetValThrAsnSerValLeuLeuAsnGlyHisSerMetLysGlnGlu

1161 ATGGCAATGTGATTTTGGCCATTCAACTCACTACCTGGAGAGAACTTAGTAA  1212
     Met:AlaMetEnd
```

TABLE 4B

This invention also encompasses proteins or peptides having substantial amino acid sequence homology with the amino acid sequences in Tables 1, 2, 3, or 4, or SEQ ID NO: 10, but excluding any protein or peptide which exhibits substantially the same or lesser amino acid sequence homology than does the substance P or substance K receptors. The substance K receptor sequence is shown in Table 6, as compared with the mouse GRP receptor.

A polypeptide "fragment", or "segment", is a stretch of amino acid residues of at least about 8 amino acids, generally at least 10 amino acids, more generally at least 12 amino acids, often at least 14 amino acids, more often at least 16 amino acids, typically at least 18 amino acids, more typically at least 20 amino acids, usually at least 22 amino acids, more usually at least 24 amino acids, preferably at least 26 amino acids, more preferably at least 28 amino acids, and, in particularly preferred embodiments, at least about 30 or more amino acids.

Amino acid sequence homology, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. This changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Homologous amino acid sequences are intended to include natural allelic and interspecies variations in each respective receptor sequence. Typical homologous proteins or peptides will have from 25-100% homology (if gaps can be introduced), to 50-100% homology (if conservative substitutions are included) with the amino acid sequence of Tables 1, 2, 3, or 4, or SEQ ID NO: 10. Homology measures will be at least about 35%, generally at least 40%, more generally at least 45%, often at least 50%, more often at least 55%, typically at least 60%, more typically at least 65%, usually at least 70%, more usually at least 75%, preferably at least 80%, and more

preferably at least 80%, and in particularly preferred embodiments, at least 85% or more. Some homologous proteins or peptides, such as the various receptor subtypes, will share various biological activities with the receptors for bombesin-like peptides of Tables 1, 2, 3, or 4, or SEQ ID NO: 10. As used herein, the term "biological activity" is defined as including, without limitation, bombesin-like protein ligand binding, cross-reactivity with antibodies raised against each respective receptor from natural sources, and coupling to guanyl nucleotide regulatory proteins (G-proteins). The G-protein linkage typically causes other functionally downstream biochemical effects including protein phosphorylation and release of sequestered Ca^{++}, both of which are often used to assay receptor function. It should be noted that various different bombesin-like peptides effect different cellular responses in the same or different cell types. A "ligand-related activity" refers either to ligand binding itself, or to biological activities which are mediated by ligand binding, including, e.g., G-protein interaction, and protein phosphorylation or Ca^{++} sequestration effects.

The term "ligand" refers to molecules, usually members of the family of bombesin-like peptides, that bind the segments involved in peptide ligand binding. Also, a ligand is a molecule which serves either as a natural ligand to which the receptor, or an analogue thereof, binds, or a molecule which is a functional analogue of a natural ligand. The functional analogue may be a ligand with structural modifications, or may be a wholly unrelated molecule which has a molecular shape which interacts with the appropriate ligand binding determinants. The ligands may serve as agonists or antagonists, see, e.g., Goodman et al. (eds) (1990) Goodman & Gilman's: The Pharmacological Bases of Therapeutics (8th ed), Pergamon Press.

Solubility of a polypeptide or fragment depends upon the environment and the polypeptide. Many parameters affect polypeptide solubility, including temperature, electrolyte environment, size and molecular characteristics of the

polypeptide, and nature of the solvent. Typically, the temperature at which the polypeptide is used ranges from about 4° C to about 65° C. Usually the temperature at use is greater than about 18° C and more usually greater than about 22° C. For diagnostic purposes, the temperature will usually be about room temperature or warmer, but less than the denaturation temperature of components in the assay. For therapeutic purposes, the temperature will usually be body temperature, typically about 37° C for humans, though under certain situations the temperature may be raised or lowered in situ or in vitro.

The electrolytes will usually approximate in situ physiological conditions, but may be modified to higher or lower ionic strength where advantageous. The actual ions may be modified to conform to standard buffers used in physiological or analytical contexts.

The size and structure of the polypeptide should generally be in a substantially stable state, and usually not in a denatured state. The polypeptide may be associated with other polypeptides in a quaternary structure, e.g., to confer solubility, or associated with lipids or detergents in a manner which approximates its natural lipid bilayer interactions.

The solvent will usually be a biologically compatible buffer, of a type used for preservation of biological activities, and will usually approximate a physiological solvent. Usually the solvent will have a neutral pH, typically between about 5 and 10, and preferably about 7.5. On some occasions, a detergent will be added, typically a mild non-denaturing one, e.g., CHS or CHAPS.

Solubility is usually measured in Svedberg units, which are a measure of the sedimentation velocity of a molecule under particular conditions. The determination of the sedimentation velocity was classically performed in an analytical ultracentrifuge, but is typically now performed in a standard ultracentrifuge. See, Freifelder (1982) Physical Biochemistry (2d ed.), W.H. Freeman; and Cantor and Schimmel (1980) Biophysical Chemistry, parts 1-3, W.H. Freeman & Co.,

San Francisco; each of which is hereby incorporated herein by reference. As a crude determination, a sample containing a putatively soluble polypeptide is spun in a standard full sized ultracentrifuge at about 50K rpm for about 10 minutes, and soluble molecules will remain in the supernatant. A soluble particle or polypeptide will typically be less than about 30S, more typically less than about 15S, usually less than about 10S, more usually less than about 6S, and, in particular embodiments, preferably less than about 4S, and more preferably less than about 3S.

II. Nucleic Acids

This invention contemplates use of isolated DNA or fragments which encode these receptors for bombesin-like peptides, e.g., each respective receptor subtype, or any fragment thereof, to encode a biologically active corresponding receptor polypeptide. In addition, this invention covers isolated or recombinant DNA which encodes a biologically active protein or polypeptide having receptor activity and which is capable of hybridizing under appropriate conditions with the DNA sequences shown in Tables 1, 2, 3, 4, or 12. Said biologically active protein or polypeptide can be a receptor itself, or fragment, and have an amino acid sequence shown in Tables 1, 2, 3, or 4, or SEQ ID NO: 10. Further, this invention covers the use of isolated or recombinant DNA, or fragments thereof, which encode proteins which are homologous to each respective receptor subtype or which was isolated using cDNA encoding a receptor for a bombesin-like peptide as a probe. The isolated DNA can have the respective regulatory sequences in the 5' and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, and others.

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other components which naturally accompany a native sequence, e.g., ribosomes, polymerases, and flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from

its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule.

An isolated nucleic acid will generally be a homogeneous composition of molecules, but will, in some embodiments, contain minor heterogeneity. This heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

A "recombinant" nucleic acid is defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence. Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants. Thus, for example, products made by transforming cells with any unnaturally occurring vector is encompassed, as are nucleic acids comprising sequence derived using any synthetic oligonucleotide process. Such is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired combination of functions not found in the commonly available natural forms. Restriction enzyme recognition sites are often the target of such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation sequences, control sequences, or other useful features may be incorporated by design. A similar concept is intended for a recombinant, e.g., fusion, polypeptide. Specifically included are synthetic nucleic acids which, by genetic code redundancy, encode similar

polypeptides to fragments of these receptors, and fusions of sequences from various different subtypes.

A "fragment" in a nucleic acid context is a contiguous segment of at least about 17 nucleotides, generally at least 20 nucleotides, more generally at least 23 nucleotides, ordinarily at least 26 nucleotides, more ordinarily at least 29 nucleotides, often at least 32 nucleotides, more often at least 35 nucleotides, typically at least 38 nucleotides, more typically at least 41 nucleotides, usually at least 44 nucleotides, more usually at least 47 nucleotides, preferably at least 50 nucleotides, more preferably at least 53 nucleotides, and in particularly preferred embodiments will be at least 56 or more nucleotides.

A DNA which codes for a receptor for a bombesin-like peptide will be particularly useful to identify genes, mRNA, and cDNA species which code for related or homologous receptors, as well as DNAs which code for receptor sub-types and receptors from different species. There is at least one receptor sub-type described with a different selectivity towards bombesin-like peptides from the subtype one which specifically binds GRP, e.g., a second subtype specific for binding NMB (subtype two), and there are likely others. In particular, a genetic sequence encoding another putative RBP has been isolated and designated "subtype three" or "R3BP", though it has not been completely characterized. Various bombesin-like peptide receptor sub-types should be highly homologous and are encompassed herein. However, even receptor proteins that have a more distant evolutionary relationship to the R1BP and do not bind gastrin releasing peptide can readily be isolated using these bombesin-like peptide receptor sequences if they are sufficiently homologous. Rat NMB receptors and human GRP and NMB receptors are examples of related receptors, as is the human R3BP. Mammalian receptors are of particular interest.

Preferred probes for such screens are those regions of the receptors which are conserved between different receptor subtypes. In particular, the third transmembrane segment,

corresponding approximately to nucleotides 345 to 410 of Table 1, is expected to show high homology to corresponding regions of other receptor subtypes. Other conserved regions will be identified by comparisons to other similar receptors or receptor subtypes, e.g., the sixth, seventh, and second transmembrane segments.

This invention further covers recombinant DNA molecules and fragments having a DNA sequence identical to or highly homologous to the isolated DNAs set forth herein. In particular, the sequences will often be operably linked to DNA segments which control transcription, translation, and DNA replication.

Homologous nucleic acid sequences, when compared, exhibit significant similarity. The standards for homology in nucleic acids are either measures for homology generally used in the art by sequence comparison or based upon hybridization conditions. The hybridization conditions are described in greater detail below, but are further limited by the homology to either of the substance P and substance K receptors. Homology measures will be limited, in addition to any stated parameters, to exceed any such similarity to the receptors for substance P or substance K.

Substantial homology in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 50% of the nucleotides, generally at least 56%, more generally at least 59%, ordinarily at least 62%, more ordinarily at least 65%, often at least 68%, more often at least 71%, typically at least 74%, more typically at least 77%, usually at least 80%, more usually at least about 85%, preferably at least about 90%, more preferably at least about 95 to 98% or more, and in particular embodiments, as high at about 99% or more of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions, to a strand, or its complement, typically using a sequence derived from Tables 1,

2, 3, 4, or 12. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14 nucleotides, preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90%. See, Kanehisa (1984) Nuc. Acids Res. 12:203-213, which is incorporated herein by reference. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 40 nucleotides, preferably at least about 50 nucleotides, and more preferably at least about 75 to 100 or more nucleotides.

Stringent conditions, in referring to homology in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters typically controlled in hybridization reactions. Stringent temperature conditions will usually include temperatures in excess of about 30° C, more usually in excess of about 37° C, typically in excess of about 45° C, more typically in excess of about 55° C, preferably in excess of about 65° C, and more preferably in excess of about 70° C. Stringent salt conditions will ordinarily be less than about 1000 mM, usually less than about 500 mM, more usually less than about 400 mM, typically less than about 300 mM, preferably less than about 200 mM, and more preferably less than about 150 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370, which is hereby incorporated herein by reference.

III. Receptor Variants

The isolated receptor DNA can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of nucleotide stretches. These modifications result in novel DNA sequences which encode these

receptors, their derivatives, or proteins having GRP receptor activity. These modified sequences can be used to produce mutant receptors or to enhance the expression of receptor species. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. Such mutant receptor derivatives include predetermined or site-specific mutations of the respective receptor or its fragments. "Mutant GRP receptor" is defined herein as encompassing a polypeptide otherwise falling within the homology definition of the GRP receptor as set forth above, but having an amino acid sequence which differs from that of GRP receptor as found in nature, whether by way of deletion, substitution or insertion. In particular, "site specific mutant GRP receptor" is defined as having homology with a receptor of Tables 1, 2, 3, or 4, or SEQ ID NO: 10, and as sharing various biological activities with those receptors. Similar concepts apply to each of the mouse and human R1BP (GRP receptor), the rat and human R2BP (NMB receptor), the human R3BP, and other receptors for bombesin-like peptides, particularly those receptors found in warm blooded animals, e.g., mammals and birds. As stated before, it is emphasized that descriptions are generally meant to encompass all receptors for bombesin-like peptides, not limited to the GRP receptor example specifically discussed.

Although site specific mutation sites are predetermined, mutants need not be site specific. GRP receptor mutagenesis can be conducted by making amino acid insertions or deletions. Substitutions, deletions, insertions, or any combinations may be generated to arrive at a final construct. Insertions include amino- or carboxy- terminal fusions. Random mutagenesis can be conducted at a target codon and the expressed GRP receptor mutants can then be screened for the desired activity. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis. See also Sambrook et al. (1989) and Ausubel et al. (1987 and Supplements).

The mutations in the DNA normally should not place coding sequences out of reading frames and preferably will not create complementary regions that could hybridize to produce secondary mRNA structure such as loops or hairpins.

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these receptors. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. Thus, the fusion product of an immunoglobulin with a receptor polypeptide is a continuous protein molecule having sequences fused in a typical peptide linkage, typically made as a single translation product and exhibiting properties derived from each source peptide. A similar concept applies to heterologous nucleic acid sequences.

In addition, new constructs may be made from combining similar functional domains from other proteins. For example, ligand-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham et al. (1989) Science 243:1330-1336; and O'Dowd et al. (1988) J. Biol. Chem. 263:15985-15992, each of which is incorporated herein by reference. Thus, new chimeric polypeptides exhibiting new combinations of specificities will result from the functional linkage of ligand-binding specificities and intracellular regions. For example, the ligand binding domains from other related receptors may be added or substituted for other binding domains of these receptors. The resulting protein will often have hybrid function and properties.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

IV. Making Receptor

DNA which encodes the GRP receptor or fragments thereof can be obtained by chemical synthesis, screening cDNA libraries, or by screening genomic libraries prepared from a wide variety of cell lines or tissue samples.

This DNA can be expressed in a wide variety of host cells for the synthesis of a full-length receptor or fragments of a receptor which can in turn, for example, be used to generate polyclonal or monoclonal antibodies; for binding studies; for construction and expression of modified receptor molecules; and for structure/function studies. Each receptor or its fragments can be expressed in host cells that are transformed or transfected with appropriate expression vectors. These molecules can be substantially free of protein or cellular contaminants, other than those derived from the recombinant host, and therefore are particularly useful in pharmaceutical compositions when combined with a pharmaceutically acceptable carrier and/or diluent. The receptor, or portions thereof, may be expressed as fusions with other proteins.

Expression vectors are typically self-replicating DNA or RNA constructs containing the desired receptor gene or its fragments, usually operably linked to suitable genetic control elements that are recognized in a suitable host cell. These control elements are capable of effecting expression within a suitable host. The specific type of control elements necessary to effect expression will depend upon the eventual host cell used. Generally, the genetic control elements can include a prokaryotic promoter system or a eukaryotic promoter expression control system, and typically include a transcriptional promoter, an optional operator to control the onset of transcription, transcription enhancers to elevate the level of mRNA expression, a sequence that encodes a suitable ribosome binding site, and sequences that terminate transcription and translation. Expression vectors also usually contain an origin of replication that allows the vector to replicate independently of the host cell.

The vectors of this invention contain DNA which encodes a receptor for a bombesin-like peptide, or a fragment thereof encoding a biologically active receptor polypeptide. The DNA can be under the control of a viral promoter and can encode a selection marker. This invention further contemplates use of such expression vectors which are capable of expressing eukaryotic cDNA coding for a receptor in a prokaryotic or eukaryotic host, where the vector is compatible with the host and where the eukaryotic cDNA coding for the receptor is inserted into the vector such that growth of the host containing the vector expresses the cDNA in question. Usually, expression vectors are designed for stable replication in their host cells or for amplification to greatly increase the total number of copies of the desirable gene per cell. It is not always necessary to require that an expression vector replicate in a host cell, e.g., it is possible to effect transient expression of the GRP receptor or its fragments in various hosts using vectors that do not contain a replication origin that is recognized by the host cell. It is also possible to use vectors that cause integration of GRP receptor or its fragments into the host DNA by recombination.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. Expression vectors are specialized vectors which contain genetic control elements that effect expression of operably linked genes. Plasmids are the most commonly used form of vector but all other forms of vectors which serve an equivalent function and which are, or become, known in the art are suitable for use herein. See, e.g., Pouwels et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., and Rodriquez et al. (eds) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Buttersworth, Boston, 1988, which are incorporated herein by reference.

Transformed cells are cells, preferably mammalian, that have been transformed or transfected with receptor vectors constructed using recombinant DNA techniques. Transformed host

cells usually express the receptor or its fragments, but for purposes of cloning, amplifying, and manipulating its DNA, do not need to express the receptor. This invention further contemplates culturing transformed cells in a nutrient medium, thus permitting the receptor to accumulate in the culture. The receptor can be recovered, either from the culture or from the culture medium.

For purposes of this invention, DNA sequences are operably linked when they are functionally related to each other. For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed as a preprotein or participates in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not contiguously linked but still bind to operator sequences that in turn control expression.

Suitable host cells include prokaryotes, lower eukaryotes, and higher eukaryotes. Prokaryotes include both gram negative and gram positive organisms, e.g., E. coli and B. subtilis. Lower eukaryotes include yeasts, e.g., S. cerevisiae and Pichia, and species of the genus Dictyostelium. Higher eukaryotes include established tissue culture cell lines from animal cells, both of non-mammalian origin, e.g., insect cells, and birds, and of mammalian origin, e.g., human, primates, and rodents.

Prokaryotic host-vector systems include a wide variety of vectors for many different species. As used herein, E. coli and its vectors will be used generically to include equivalent vectors used in other prokaryotes. A representative vector for amplifying DNA is pBR322 or many of its derivatives. Vectors that can be used to express the receptor or its fragments include, but are not limited to, such vectors as those containing the lac promoter (pUC-series); trp promoter

(pBR322-trp); Ipp promoter (the pIN-series); lambda-pP or pR promoters (pOTS); or hybrid promoters such as ptac (pDR540). See Brosius et al. (1988) "Expression Vectors Employing Lambda-, trp-, lac-, and Ipp-derived Promoters", in Vectors: A Survey of Molecular Cloning Vectors and Their Uses, (eds. Rodriguez and Denhardt), Buttersworth, Boston, Chapter 10, pp. 205-236, which is incorporated herein by reference.

Lower eukaryotes, e.g., yeasts and Dictyostelium, may be transformed with GRP receptor sequence containing vectors. For purposes of this invention, the most common lower eukaryotic host is the baker's yeast, Saccharomyces cerevisiae. It will be used to generically represent lower eukaryotes although a number of other strains and species are also available. Yeast vectors typically consist of a replication origin (unless of the integrating type), a selection gene, a promoter, DNA encoding the receptor or its fragments, and sequences for translation termination, polyadenylation, and transcription termination. Suitable expression vectors for yeast include such constitutive promoters as 3-phosphoglycerate kinase and various other glycolytic enzyme gene promoters or such inducible promoters as the alcohol dehydrogenase 2 promoter or metallothionine promoter. Suitable vectors include derivatives of the following types: self-replicating low copy number (such as the YRp-series), self-replicating high copy number (such as the YEp-series); integrating types (such as the YIp-series), or mini-chromosomes (such as the YCp-series).

Higher eukaryotic tissue culture cells are the preferred host cells for expression of the functionally active GRP receptor protein. In principle, any higher eukaryotic tissue culture cell line is workable, e.g., insect baculovirus expression systems, whether from an invertebrate or vertebrate source. However, mammalian cells are preferred. Transformation or transfection and propagation of such cells has become a routine procedure. Examples of useful cell lines include HeLa cells, Chinese hamster ovary (CHO) cell lines, baby rat kidney (BRK) cell lines, insect cell lines, bird cell lines, and monkey (COS) cell lines. Expression vectors for

such cell lines usually include an origin of replication, a promoter, a translation initiation site, RNA splice sites (if genomic DNA is used), a polyadenylation site, and a transcription termination site. These vectors also usually contain a selection gene or amplification gene. Suitable expression vectors may be plasmids, viruses, or retroviruses carrying promoters derived, e.g., from such sources as from adenovirus, SV40, parvoviruses, vaccinia virus, or cytomegalovirus. Representative examples of suitable expression vectors include pCDNA1; pCD, see Okayama et al. (1985) Mol. Cell Biol. 5:1136-1142; pMC1neo PolyA, see Thomas et al. (1987) Cell 51:503-512; and a baculovirus vector such as pAC 373 or pAC 610.

It will often be desired to express a receptor polypeptide in a system which provides a specific or defined glycosylation pattern. In this case, the usual pattern will be that provided naturally by the expression system. However, the pattern will be modifiable by exposing the polypeptide, e.g., an unglycosylated form, to appropriate glycosylating proteins introduced into a heterologous expression system. For example, the receptor gene may be co-transformed with one or more genes encoding mammalian or other glycosylating enzymes. Using this approach, certain mammalian glycosylation patterns will be achievable in prokaryote or other cells.

V. Receptor Isolation

The GRP receptor can be solubilized from membranes in an active form, and purified without loss of activity by the methods outlined below. Again, although the methods are applied to GRP receptor, other receptors for bombesin-like peptides will behave similarly and should be isolatable using analogous methods.

The source of GRP receptor can be a eukaryotic or prokaryotic host expressing recombinant GRP receptor DNA, such as is described above. The source can also be a cell line such as mouse Swiss 3T3 fibroblasts, but other mammalian cell lines

are also contemplated by this invention, with the preferred cell line being from the human species.

The active GRP receptor was solubilized from membranes containing the GRP receptor using a stabilizing agent and a detergent. The stabilizing agent is preferably a soluble cholesteryl ester. Particularly good results have been obtained using cholesteryl hemisuccinate (CHS). The detergent can be non-ionic, zwitter-ionic, or the like. Particularly good results have been obtained using the zwitter-ionic detergent 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (CHAPS).

Cellular membranes containing the GRP receptor are prepared by lysis of a cultured GRP receptor containing cell line, e.g., Swiss 3T3 fibroblasts, followed by centrifugation. The resulting pellets are washed by resuspension and centrifuged again.

Once the membranes are obtained from a suitable cell line, as described above and in Example 1, the final concentration of protein is adjusted. A suitable final protein concentration is about 15 mg/ml.

The membranes are then salt washed prior to solubilization of the GRP receptor. The membranes are washed twice with buffer and sodium chloride (NaCl), then washed with a solubilization buffer and finally suspended in the solubilization buffer at an adjusted protein concentration. A suitable buffer composition for the first two washings comprises a medium such as 50 mM 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES), pH 7.5, a chelator such as 2 mM ethylenediamine-tetraacetic acid (EDTA), and protease inhibitors. A suitable NaCl concentration is 1.0 M. The solubilization buffer, both for the washing and suspension, can be typically comprised of 50 mM HEPES, pH 7.5, 2 mM EDTA, another chelator such as 1 mM [ethylenebis-(oxyethylenenitrilo)]tetraacetic acid (EGTA), 100 mM NaCl, and protease inhibitors. The protein concentration is adjusted to about 7 mg/ml, for example. This salt washing step provides a two-fold purification. Similar results can be

achieved by washing the membranes with 2 M urea, high pH buffers (pH 10), or chaotropic salts, e.g., potassium iodide (KI). This procedure also increases the stability of the GRP receptor in the extract. Other constituents of the buffers may include, e.g., sucrose, and suitable protease inhibitors include, without limitation, aprotinin, leupeptin, pepstatin, bacitrin, and phenylmethylsulfonyl fluoride (PMSF).

A mixture of detergent (CHAPS) and soluble cholesteryl ester stabilizing agent (CHS) is then slowly added to the membrane suspension to give a set final detergent concentration. The weight ratio of detergent to soluble cholesteryl ester can be within the range of about 200:1 to 5:2, preferably about 10:1. Alternatively, the detergent can be added to the membrane suspension, followed by the addition of the soluble cholesteryl ester. In that instance, initially there will be 100% detergent and the soluble cholesteryl ester is added until the weight ratio of detergent to ester is within the range of about 200:1 to 5:2, preferably about 10:1. For solubilization of the GRP receptor, the concentration of detergent should be 0.4 to 3.0% weight per volume (w/v), and is optimally set at about 0.75% (w/v) for a membrane concentration (prior to the membrane washing steps) of around 15 mg/ml. Similarly, the concentration of soluble cholesteryl ester is within the range of about 0.0015 to 1.2% (w/v). Likewise, for a membrane concentration of around 15 mg/ml, the concentration of soluble cholesteryl ester is preferably about 0.075% (w/v).

The extract is then incubated at a temperature within the range of about 0 to 37° C, typically at room temperature such as 21° C, and then cooled to 0 to 21° C, typically 4° C. The insoluble material is then centrifuged at high speeds, preferably about 100,000 times gravity, in a standard centrifuge for a suitable period of time, depending upon the volume involved, to obtain an extract containing the solubilized receptor (i.e., soluble extract).

At high detergent concentration (0.4 to 3.0%), the receptor loses biological activity. However, upon dilution with a buffer solution, the receptor is reactivated. The

presence of the soluble cholesteryl ester, which acts as a stabilizing agent, is necessary for the receptor to be reactivated at the low detergent concentration. For assays using the active solubilized GRP receptor to exhibit binding activity, the final concentration of detergent in the suspension should be diluted to within the range of about 0.025 to 0.2% (w/v). The weight ratio of detergent to soluble cholesteryl ester is still maintained within the range of about 200:1 to 5:2, preferably about 10:1. Therefore, a suitable range for the soluble cholesteryl ester is about 0.000125 to 0.08% (w/v). The preferable assay concentrations are 0.075% (w/v) detergent and about 0.0075% (w/v) soluble cholesteryl ester.

The solubilized receptor in its active form is then purified and freed of contaminating proteins. Purification of the GRP receptor involves a multistep procedure which includes the following steps, which follow the solubilization procedure as set forth above.

(1) Polyethylene glycol precipitation. The GRP receptor is precipitated from the soluble extract by addition of polyethylene glycol (PEG). Addition of PEG is preferably done to obtain a final concentration of 20% (w/v). The precipitate is then collected by centrifugation and resuspended in a buffer solution. The buffer solution can typically be comprised of 25 mM HEPES, pH 7.5, 25 mM TRIS/Cl, 2 mM EDTA, 0.075% (w/v) detergent, 0.0075% (w/v) soluble cholesteryl ester, and protease inhibitors. The final volume of the suspension is preferably 25% that of the original soluble extract. Proteins remaining insoluble in the suspension are removed by centrifugation. This step provides a two-fold purification, and enhances the stability of the receptor.

(2) Wheat germ agglutinin chromatography. The soluble extract is applied to a wheat germ agglutinin affinity column equilibrated with a buffer solution typically comprised of 50 mM HEPES, pH 7.5, 2 mM EDTA, 0.25% (w/v) detergent, 0.025% (w/v) cholesteryl ester, and protease inhibitors. The column is eluted with column buffer solution and 5 mM

N-N'-N"-triacetyl-chitotriose. Fractions containing the GRP receptor are then identified by ^{125}I-GRP binding assays. This step provides a five-fold purification by removing proteins that do not contain carbohydrate. To obtain a good yield, it is necessary to elute the column with chitotriose or chitobiose. The yield may also be enhanced by maintaining the detergent concentration above about 0.2% detergent and 0.02% soluble cholesteryl ester.

(3) GRP-affinity chromatography. The wheat germ agglutinin column eluate is further fractionated on a GRP affinity column. In the preferred embodiment, the column contains a beaded matrix with the peptide human [Nle14,27]GRP13-27 (the C-terminal portion of GRP) coupled to it at 2 mg peptide/ml packed gel. The column is equilibrated with a solution typically comprised of 25 mM TRIS, 25 mM HEPES, pH 7.5, 2 mM EDTA, 0.075% (w/v) CHAPS, 0.0075% (w/v) CHS, and protease inhibitors. The concentration of detergent in the wheat germ agglutinin column eluate is preferably adjusted to 0.075% (w/v) by dilution with a solution typically comprised of 25 mM HEPES, 25 mM TRIS, pH 7.5, 2 mM EDTA, and protease inhibitors. After application of the sample and extensive washing of the column, bound protein is eluted with a salt at a concentration above 0.2 M. Particularly suitable is 0.5 M NaCl. Fractions containing the GRP receptor are then identified by ^{125}I-GRP binding assays. The GRP peptide used ([Nle14,27]GRP13-27) is an analog made by Triton Biosciences Inc. (Alameda, CA) which is resistant to oxidation. Other GRP peptides and matrixes that will also work include, without limitation, GRP1-27, GRP14-27, and [Lys3]Bombesin, though the optimum yield and elution conditions may involve adjustment. Elution of the bound protein with salt is important because receptor binding activity is preserved and a good yield is achieved. The concentration of detergent in the sample loaded onto the column is carefully optimized. The suitable range of detergent is about 0.025 to 0.2% (w/v). The ratio of detergent to stabilizing agent is also the same, being 200:1 to 5:2, preferably 10:1.

(4) Second affinity column. Fractions containing the GRP receptor eluted from the affinity column are desalted and the sample is applied to a second GRP affinity column, and eluted as described in step (3). Fractions containing the receptor are then identified by binding assays. Use of two consecutive affinity columns in this step is preferred to give a high degree of purity.

(5) Gel filtration. This is an optional step that yields a purer product. The gel filtration step is also useful to remove protease inhibitors, salt, and residual detergent from the receptor.

In general, the solubilized, unpurified and solubilized, purified GRP receptor of this invention binds gastrin releasing peptide with an affinity of at least K_D=10 nM. The GRP receptor from a mouse Swiss 3T3 fibroblast cell line, according to this invention was found to have the following characteristics: runs as a broad band on SDS-PAGE with an apparent molecular weight of about 70 to 100 kilodaltons; binds selectively with polypeptides of the bombesin type; has a K_D value of about 10-100 pM; is free of coupled G proteins; contains N-linked carbohydrates; when deglycosylated, has an apparent molecular weight of 36±5 kilodaltons on SDS-PAGE; and has a partial amino acid sequence near the N-terminus of:

-Leu-Asn-Leu-Asp-Val-Asp-Pro-Phe-Leu-Ser-.

Now that the entire sequence is known, the GRP receptor, fragments or derivatives thereof can be prepared by conventional processes for synthesizing peptides. These include processes such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL; Bodanszky and Bodanszky (1984) The Practice of Peptide Synthesis, Springer-Verlag, New York; and Bodanszky (1984) The Principles of Peptide Synthesis, Springer-Verlag, New York; all of each which are incorporated herein by reference. For example, an azide process, an acid chloride process, an acid anhydride process, a mixed anhydride process, an active ester process (for example, p-nitrophenyl ester,

N-hydroxysuccinimide ester, or cyanomethyl ester), a carbodiimidazole process, an oxidative-reductive process, or a dicyclohexylcarbodiimide (DCCD)/additive process can be used. Solid phase and solution phase syntheses are both applicable to the foregoing processes.

The GRP receptor, fragments or derivatives are suitably prepared in accordance with the above processes as typically employed in peptide synthesis, generally either by a so-called stepwise process which comprises condensing an amino acid to the terminal amino acid, one by one in sequence, or by coupling peptide fragments to the terminal amino acid. Amino groups that are not being used in the coupling reaction must be protected to prevent coupling at an incorrect location.

If a solid phase synthesis is adopted, the C-terminal amino acid is bound to an insoluble carrier or support through its carboxyl group. The insoluble carrier is not particularly limited as long as it has a binding capability to a reactive carboxyl group. Examples of such insoluble carriers include halomethyl resins, such as chloromethyl resin or bromomethyl resin, hydroxymethyl resins, phenol resins, tert-alkyloxycarbonylhydrazidated resins, and the like.

An amino group-protected amino acid is bound in sequence through condensation of its activated carboxyl group and the reactive amino group of the previously formed peptide or chain, to synthesize the peptide step by step. After synthesizing the complete sequence, the peptide is split off from the insoluble carrier to produce the peptide. This solid-phase approach is generally described by Merrifield et al. (1963) in J. Am. Chem. Soc. 85:2149-2156, which is incorporated herein by reference.

The prepared receptor and fragments thereof can be isolated and purified from the reaction mixture by means of peptide separation, for example, by extraction, precipitation, electrophoresis and various forms of chromatography, and the like. The receptor of this invention can be obtained in varying degrees of purity depending upon its desired use. Purification can be accomplished by use of the protein

purification techniques disclosed herein or by the use of the antibodies herein described in immunoabsorbant affinity chromatography. This immunoabsorbant affinity chromatography is carried out by first linking the antibodies to a solid support and then contacting the linked antibodies with solubilized lysates of small cell lung cancer cells, lysates of other cells expressing the GRP receptor, or lysates or supernatants of cells producing the GRP receptor as a result of DNA techniques, see below.

VI. Receptor Analogues

"Derivatives" of the GRP receptor include amino acid sequence mutants, glycosylation variants, and covalent or aggregative conjugates with other chemical moieties. Covalent derivatives can be prepared by linkage of functionalities to groups which are found in the GRP receptor amino acid side chains or at the N- or C- termini, by means which are well known in the art. These derivatives can include, without limitation, aliphatic esters or amides of the carboxyl terminus, or of residues containing carboxyl side chains, O-acyl derivatives of hydroxyl group-containing residues, and N-acyl derivatives of the amino terminal amino acid or amino-group containing residues, e.g., lysine or arginine. Acyl groups are selected from the group of alkyl-moieties including C3 to C18 normal alkyl, thereby forming alkanoyl aroyl species.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. Particularly preferred means for accomplishing this are by exposing the polypeptide to glycosylating enzymes derived from cells which normally provide such processing, e.g., mammalian glycosylation enzymes. Deglycosylation enzymes are also contemplated. Also embraced are versions of the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid

residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

A major group of derivatives are covalent conjugates of the GRP receptor or fragments thereof with other proteins of polypeptides. These derivatives can be synthesized in recombinant culture such as N- or C-terminal fusions or by the use of agents known in the art for their usefulness in cross-linking proteins through reactive side groups. Preferred GRP derivatization sites with cross-linking agents are at free amino groups, carbohydrate moieties, and cysteine residues.

Fusion polypeptides between the receptors and other homologous or heterologous proteins are also provided. Homologous polypeptides may be fusions between different growth factor receptors, resulting in, for instance, a hybrid protein exhibiting ligand specificity of one receptor and the intracellular region of another, or a receptor which may have broadened or weakened specificity of binding. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. Typical examples are fusions of a reporter polypeptide, e.g., luciferase, with a segment or domain of a receptor, e.g., a ligand-binding segment, so that the presence or location of a desired ligand may be easily determined. See, e.g., Dull et al., U.S. Patent No. 4,859,609, which is hereby incorporated herein by reference. Other gene fusion partners include bacterial β-galactosidase, trpE, Protein A, β-lactamase, alpha amylase, alcohol dehydrogenase, and yeast alpha mating factor. See, e.g,., Godowski et al. (1988) Science 241:812-816.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Such polypeptides may also have amino acid residues which have been chemically modified by phosphorylation, sulfonation, biotinylation, or the addition or removal of other moieties, particularly those which have molecular shapes similar to phosphate groups. In some embodiments, the modifications will be useful labeling reagents, or serve as purification targets, e.g., affinity ligands.

Fusion proteins will typically be made by either recombinant nucleic acid methods or by synthetic polypeptide methods. Techniques for nucleic acid manipulation and expression are described generally, for example, in Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.), Vols. 1-3, Cold Spring Harbor Laboratory, which are incorporated herein by reference. Techniques for synthesis of polypeptides are described, for example, in Merrifield (1963) J. Amer. Chem. Soc. 85:2149-2156; Merrifield (1986) Science 232: 341-347; and Atherton et al. (1989) Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, Oxford; each of which is incorporated herein by reference.

This invention also contemplates the use of derivatives of the GRP receptor other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties. These derivatives generally fall into three classes: (1) salts, (2) side chain and terminal residue covalent modifications, and (3) adsorption complexes, for example with cell membranes. Such covalent or aggregative derivatives are useful as immunogens, as reagents in immunoassays or in purification methods such as for affinity purification of gastrin releasing peptide or other binding ligands. For example, the GRP receptor can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated Sepharose, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of anti-GRP receptor antibodies or gastrin releasing peptide. The GRP receptor can also be labeled with a detectable group, for

example radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates, or conjugated to another fluorescent moiety for use in diagnostic assays.

The solubilized GRP receptor of this invention can be used as an immunogen for the production of antisera or antibodies specific for the receptor or any fragments thereof. The purified receptor can be used to screen monoclonal antibodies or antigen-binding fragments prepared by immunization with various forms of impure preparations containing the GRP receptor. In particular, the term "antibodies" also encompasses antigen binding fragments of natural antibodies. The purified receptor can also be used as a reagent to detect any antibodies generated in response to the presence of elevated levels of gastrin releasing peptide receptor or cell fragments containing the GRP receptor. Additionally, GRP receptor fragments may also serve as immunogens to produce the antibodies of the present invention, as described immediately below. For example, this invention contemplates antibodies having binding affinity to or being raised against the amino acid sequence shown in Tables 1, 2, 3, or 4, or SEQ ID NO: 10, or fragments thereof. In particular, this invention contemplates antibodies having binding affinity to or being raised against specific fragments which are predicted to lie outside of the lipid bilayer. These fragments include the following ten amino acid sequence (residues 9-18, inclusive) near the N-terminus:

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          9                                   18
-Leu-Asn-Leu-Asp-Val-Asp-Pro-Phe-Leu-Ser-
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In addition, this invention covers fragments of the GRP receptor which are predicted to reside on the extracellular side of the membrane: residues 1-39, inclusive; residues 98-115, inclusive; residues 176-209, inclusive; and residues 288-300, inclusive; and to the following portions of the receptor which are predicted to reside on the intracellular side of the membrane: residues 64-77, inclusive; residues 138-157, inclusive; residues 236-266, inclusive; and residues

330-385, inclusive. Analogous regions of other receptors for bombesin-like peptides will also be used.

VII. Antibodies

Antibodies can be raised to the various subtypes of RBP, e.g., GRP and related receptors, and fragments thereof, both in their naturally occurring forms and in their recombinant forms. Additionally, antibodies can be raised to GRP receptors in either their active forms or in their inactive forms, the difference being that antibodies to the active receptor are more likely to recognize epitopes which are only present in the active receptor. Anti-idiotypic antibodies are also contemplated.

Antibodies, including binding fragments and single chain versions, against predetermined fragments of the GRP receptor can be raised by immunization of animals with conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective GRP receptors, or screened for agonistic or antagonistic GRP receptor activity. These monoclonal antibodies will usually bind with at least a K_D of about 1 mM, more usually at least about 300 μM, typically at least about 10 μM, more typically at least about 30 μM, preferably at least about 10 μM, and more preferably at least about 3 μM or better. Although the foregoing addresses GRP receptors, similar antibodies will be raised against other receptors, or receptor subtypes, for bombesin-like peptides.

The antibodies, including antigen binding fragments, of this invention can have significant diagnostic or therapeutic value. They can be potent antagonists that bind to the GRP receptor and inhibit ligand binding to the receptor or inhibit the ability of gastrin releasing peptide to elicit a biological response. They also can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides so that when the antibody binds to the receptor, the cell itself is killed. Further, these antibodies can be

conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker.

The antibodies of this invention can also be useful in diagnostic applications. As capture or non-neutralizing antibodies, they can bind to the GRP receptor without inhibiting ligand binding. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying GRP or GRP receptors.

Receptor fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as immunogens. The GRP receptor and its fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. See Microbiology, Hoeber Medical Division, Harper and Row, 1969; Landsteiner (1962) Specificity of Serological Reactions, Dover Publications, New York, and Williams et al. (1967) Methods in Immunology and Immunochemistry, Vol. 1, Academic Press, New York, each of which are incorporated herein by reference, for descriptions of methods of preparing polyclonal antisera. A typical method involves hyperimmunization of an animal with an antigen. The blood of the animal is then collected shortly after the repeated immunizations and the gamma globulin is isolated.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, e.g., Stites et al. (eds) Basic and Clinical Immunology (4th ed.), Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed) Academic Press, New York; and particularly in Kohler and Milstein (1975) in Nature 256: 495-497, which discusses one method of generating monoclonal antibodies. Each of these references is incorporated herein by reference. Summarized briefly, this method involves injecting an animal with an

immunogen. The animal is then sacrificed and cells taken from its spleen, which are then fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing in vitro. The population of hybridomas is then screened to isolate individual clones, each of which secrete a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic substance.

Other suitable techniques involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors. See, Huse et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science 246:1275-1281; and Ward et al. (1989) Nature 341:544-546, each of which is hereby incorporated herein by reference. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents, teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced, see Cabilly, U.S. Patent No. 4,816,567. These patents are incorporated herein by reference.

The antibodies of this invention can also be used for affinity chromatography in isolating the receptor. Columns can be prepared where the antibodies are linked to a solid support, e.g., particles, such as agarose, Sephadex, or the like, where a cell lysate may be passed through the column, the column

washed, followed by increasing concentrations of a mild denaturant, whereby the purified receptor protein will be released.

The antibodies may also be used to screen expression libraries for particular expression products. Usually the antibodies used in such a procedure will be labeled with a moiety allowing easy detection of presence of antigen by antibody binding.

Antibodies raised against each receptor will also be used to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the respective receptors.

VIII. Other Uses of Receptors

Both the naturally occurring and the recombinant form of the receptors for bombesin-like peptides of this invention are particularly useful in kits and assay methods which are capable of screening compounds for binding activity to the receptors. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds per year. See, e.g, Fodor et al. (1991) Science 251:767-773, which is incorporated herein by reference and which describes means for testing of binding affinity by a plurality of defined polymers synthesized on a solid substrate. The development of suitable assays can be greatly facilitated by the availability of large amounts of purified, soluble receptor in an active state such as is provided by this invention.

For example, antagonists can normally be found once the receptor has been pharmacologically defined, as is the case now with the GRP and NMB receptors. Testing of potential receptor antagonists is now possible upon the development of highly automated assay methods using a purified receptor. In particular, new agonists and antagonists will be discovered by using screening techniques made available herein. Of particular importance are compounds found to have a combined binding affinity for multiple receptor subtypes, e.g.,

compounds which can serve as antagonists for both a GRP receptor and a NMB receptor. Such compounds provide methods for simultaneously affecting multiple receptor subtypes.

This invention is particularly useful for screening compounds by using the recombinant receptors in any of a variety of drug screening techniques. The advantages of using a recombinant receptor in screening for receptor reactive drugs include: (a) improved renewable source of the receptor from a specific source; (b) potentially greater number of receptors per cell giving better signal to noise ratio in assays; and (c) receptor subtype specificity (theoretically giving greater biological and disease specificity).

One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing the a receptor. Cells may be isolated which express a single receptor subtype insolation from any others. Such cells, either in viable or fixed form, can be used for standard receptor/ligand binding assays. See also, Parce et al. (1989) Science 246:243-247; and Owicki et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011, which are incorporated herein by reference and describe sensitive methods to detect cellular responses. Competitive assays are particularly useful, where the cells (source of RBP) are contacted and incubated with a labeled ligand having known binding affinity to the receptor, such as ^{125}I-GRP, and a test compound whose binding affinity to the GRP receptor is being measured. The bound ligand and free ligand are then separated to assess the degree of ligand binding. The amount of test compound bound is inversely proportional to the amount of labeled ligand binding measured. Any one of numerous techniques can be used to separate bound from free ligand to assess the degree of ligand binding. This separation step could typically involve a procedure such as adhesion to filters followed by washing, adhesion to plastic followed by washing, or centrifugation of the cell membranes. Viable cells could also be used to screen for the effects of drugs on GRP receptor mediated functions, e.g., second messenger levels, i.e., Ca^{++};

cell proliferation; inositol phosphate pool changes; and others. Some detection methods allow for elimination of a separation step, e.g., a proximity sensitive detection system. Calcium sensitive dyes will be useful for detecting Ca^{++} levels, with a fluorimeter or a fluorescence cell sorting apparatus.

Another method utilizes membranes from transformed eukaryotic or prokaryotic host cells as the source of the GRP receptor. These cells are stably transformed with DNA vectors directing the expression of the GRP receptor. Essentially, the membranes would be prepared from the cells and used in any receptor/ligand binding assay such as the competitive assay set forth above.

Still another approach is to use solubilized, unpurified or solubilized, purified receptors from transformed eukaryotic or prokaryotic host cells. This allows for a "molecular" binding assay with the advantages of increased specificity, the ability to automate, and high drug test throughput.

Another technique for drug screening involves an approach which provides high throughput screening for compounds having suitable binding affinity to the gastrin- releasing peptide receptor and is described in detail in Geysen, European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference. First, large numbers of different small peptide test compounds are synthesized on a solid substrate, e.g., plastic pins or some other appropriate surface, see Fodor et al. (1991). Then all the pins are reacted with solubilized, unpurified or solubilized, purified GRP receptor, and washed. The next step involves detecting bound GRP receptor.

Rational drug design may also be based upon structural studies of the molecular shapes of the receptor and other effectors or ligands. Effectors may be other proteins which mediate other functions in response to ligand binding, or other proteins which normally interact with the receptor. One means for determining which sites interact with specific other

proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional NMR techniques. These will provide guidance as to which amino acid residues form the molecular contact regions. For a detailed description of protein structural determination, see, e.g., Blundell and Johnson (1976) Protein Crystallography, Academic Press, New York, which is hereby incorporated herein by reference.

Purified receptor can be coated directly onto plates for use in the aforementioned drug screening techniques. However, non-neutralizing antibodies to these receptors can be used as capture antibodies to immobilize the respective receptor on the solid phase.

IX. Ligands: Agonists and Antagonists

The blocking of physiological response to bombesin-like peptides may result from the inhibition of binding of the ligand to the receptor, likely through competitive inhibition. Thus, in vitro assays of the present invention will often use isolated membranes from cells expressing a recombinant receptor, soluble fragments comprising the ligand binding segments of these receptors, or fragments attached to solid phase substrates. These assays will also allow for the diagnostic determination of the effects of either binding segment mutations and modifications, or ligand mutations and modifications, e.g., ligand analogues.

This invention also contemplates the use of competitive drug screening assays, e.g., where neutralizing antibodies to the receptor or receptor fragments compete with a test compound for binding to the receptor. In this manner, the antibodies can be used to detect the presence of any polypeptide which shares one or more binding sites of the receptor and can also be used to occupy binding sites on the receptor that might otherwise be occupied by a bombesin-like peptide.

Additionally, neutralizing antibodies against the receptor and soluble fragments of the receptor which contain the high affinity ligand binding site, can be used to inhibit

gastrin releasing peptide receptor function in cancerous tissues, e.g., tissues experiencing proliferative abnormalities.

X. Kits

This invention also contemplates use of the GRP receptor, fragments thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for detecting the presence of the gastrin releasing peptide receptor. Typically the kit will have a compartment containing either a defined receptor peptide or gene segment or a reagent which recognizes one or the other.

A kit for determining the binding affinity of a test compound to the gastrin releasing peptide receptor would typically comprise a test compound; a labeled compound, for example a ligand or antibody having known binding affinity for the gastrin releasing peptide receptor; a source of gastrin releasing peptide receptor (naturally occurring or recombinant); and a means for separating bound from free labeled compound, such as a solid phase for immobilizing the gastrin releasing peptide receptor. Once compounds are screened, those having suitable binding affinity to the GRP receptor can be evaluated in suitable biological assays, as are well known in the art, to determine whether they act as agonists or antagonists. The availability of recombinant receptor polypeptides also provide well defined standards for calibrating such assays.

A preferred kit for determining the concentration of, for example, gastrin releasing peptide receptor in a sample would typically comprise a labeled compound, e.g., ligand or antibody, having known binding affinity for the gastrin releasing peptide receptor, a source of gastrin releasing peptide receptor (naturally occurring or recombinant) and a means for separating the bound from free labeled compound, for example a solid phase for immobilizing the gastrin releasing peptide receptor. Compartments containing reagents, and instructions, will normally be provided.

One method for determining the concentration of gastrin-releasing peptide receptor in a sample would typically comprise the steps of: (1) preparing membranes from a sample comprised of a GRP receptor source; (2) washing the membranes and suspending them in a buffer; (3) solubilizing the GRP receptor by incubating the membranes in a culture medium to which a detergent and a soluble cholesteryl ester has been added; (4) adjusting the detergent concentration of the solubilized receptor; (5) contacting and incubating said dilution with radiolabeled GRP to form GRP:GRP receptor complexes; (6) recovering the complexes such as by filtration through polyethyleneimine treated filters; and (7) measuring the radioactivity of the recovered complexes. Similar methods should be applicable to other members of the family of RBP.

Antibodies, including antigen binding fragments, specific for the receptor or receptor fragments are useful in diagnostic applications to detect the presence of elevated levels of the receptor and/or its fragments. Such diagnostic assays can employ lysates, live cells, fixed cells, immunofluorescence, cell cultures, body fluids, and further can involve the detection of antigens related to the GRP receptor in serum, or the like. Diagnostic assays may be homogeneous (without a separation step between free reagent and receptor-ligand complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA) and the like. For example, unlabeled antibodies can be employed by using a second antibody which is labeled and which recognizes the antibody to the GRP receptor or to a particular fragment thereof. These assays have also been extensively discussed in the literature. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH.

Anti-idiotypic antibodies may have similar use to diagnose presence of antibodies against a receptor, as such may be diagnostic of various abnormal states. For example,

overproduction of RBP may result in production of various immunological reactions which may be diagnostic of abnormal receptor expression, particularly in proliferative cell conditions such as cancer.

Frequently, the reagents for diagnostic assays are supplied in kits, so as to optimize the sensitivity of the assay. For the subject invention, depending upon the nature of the assay, the protocol, and the label, either labeled or unlabeled antibody, or labeled receptor is provided. This is usually in conjunction with other additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions for proper use and disposal of the contents after use. Typically the kit has compartments for each useful reagent. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium having appropriate concentrations for performing the assay.

Any of the aforementioned constituents of the drug screening and the diagnostic assays may be used without modification or may be modified in a variety of ways. For example, labeling may be achieved by covalently or non-covalently joining a moiety which directly or indirectly provides a detectable signal. In any of these assays, the ligand, test compound, GRP receptor, or antibodies thereto can be labeled either directly or indirectly. Possibilities for direct labeling include label groups: radiolabels such as ^{125}I, enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Both of the patents are incorporated herein by reference. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating the bound from the free ligand, or alternatively the bound from the

free test compound. The receptor can be immobilized on various matrixes followed by washing. Suitable matrixes include plastic such as an ELISA plate, filters, and beads. Methods of immobilizing the receptor to a matrix include, without limitation, direct adhesion to plastic, use of a capture antibody, chemical coupling, and biotin-avidin. The last step in this approach involves the precipitation of receptor/ligand complex by any of several methods including those utilizing, e.g., an organic solvent such as polyethylene glycol or a salt such as ammonium sulfate. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in Rattle et al. (1984) Clin. Chem. 30(9):1457-1461, and the double antibody magnetic particle separation as described in U.S. Pat. No. 4,659,678, each of which is incorporated herein by reference.

The methods for linking protein receptors or their fragments to the various labels have been extensively reported in the literature and do not require detailed discussion here. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an activated halogen such as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins will also find use in these applications.

Another diagnostic aspect of this invention involves use of oligonucleotide or polynucleotide sequences taken from the sequence of a receptor for GRP or other bombesin-like peptide. These sequences can be used as probes for detecting levels of the receptor in patients suspected of having a proliferative cell conditions, e.g., cancer. The preparation of both RNA and DNA nucleotide sequences, the labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature. Normally an oligonucleotide probe should have at least about 14 nucleotides, usually at least about 18 nucleotides, and the polynucleotide probes may be up to several kilobases. Various

labels may be employed, most commonly radionuclides, particularly ^{32}P. However, other techniques may also be employed, such as using biotin modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed which can recognize specific duplexes, including DNA duplexes, RNA duplexes, DNA-RNA hybrid duplexes, or DNA-protein duplexes. The antibodies in turn may be labeled and the assay carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected. The use of probes to the novel anti-sense RNA may be carried out in any conventional techniques such as nucleic acid hybridization, plus and minus screening, recombinational probing, hybrid released translation (HRT), and hybrid arrested translation (HART). This also includes amplification techniques such as polymerase chain reaction (PCR).

Diagnostic kits which also test for the qualitative or quantitative presence of other markers are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet et al. (1989) Progress in Growth Factor Res. 1:89-97.

Similar reagents are made available for application of these concepts to receptors for other bombesin-like peptides.

XI. Therapeutic Applications

This invention provides reagents with significant therapeutic value. The GRP receptor (naturally occurring or recombinant), fragments thereof and antibodies thereto, along with compounds identified as having binding affinity to the GRP receptor, should be useful in the treatment of conditions exhibiting proliferative growth, e.g., cancerous tissues, such as prostatic and pancreatic tumors, and particularly in the

treatment of small cell lung cancer. Additionally, this invention should have therapeutic value in any disease or disorder associated with abnormal expression or abnormal triggering of receptors for GRP or other bombesin-like peptides. For example, it is believed that the GRP receptor likely plays a role in neurologic function, and can affect gastrointestinal, pulmonary, and brain tissue. As before, the basic principles underlying the descriptions here directed towards GRP receptors will also be applicable to other receptors for bombesin-like peptides.

Recombinant GRP receptor or GRP receptor antibodies can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, along with physiologically innocuous stabilizers and excipients. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof which are not complement binding.

Drug screening using the GRP receptor or fragments thereof can be performed to identify compounds having binding affinity to the GRP receptor. Subsequent biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of gastrin releasing peptide. Likewise, a compound having intrinsic stimulating activity can activate the receptor and is thus an agonist in that it simulates the activity of gastrin releasing peptide. This invention further contemplates the therapeutic use of antibodies to the GRP receptor as antagonists. This approach should be particularly useful with other receptors for bombesin-like peptides. For example effective antagonists for the NMB receptor have not been found, and identification of a ligand for the R3BP has not yet been done.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman et al. (eds) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; each of which is hereby incorporated herein by reference. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. Because of the high affinity binding between a bombesin-like peptide and its receptors, low dosages of these reagents would be initially expected to be effective. Thus, dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μM concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or slow release apparatus will often be utilized for continuous administration. The intracellular segments of the receptors, both the GRP receptor and related receptors will find additional uses as described in detail below.

The GRP receptor, fragments thereof, and antibodies to the receptor or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to

conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in any conventional dosage formulation. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier must be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman et al. (eds) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; each of which is hereby incorporated herein by reference. The therapy of this invention may be combined with or used in association with other chemotherapeutic or chemopreventive agents.

XII. Receptor Subtypes

The present invention contemplates the isolation of additional closely related receptors for other bombesin-like peptides. As described above, these are various types of bombesin-like peptides having different functions. See, e.g., LeBacq-Verheyden et al. (1990), which is incorporated herein by reference. Various of these peptides have been functionally classified as digestive hormones, central modulators of metabolism, growth factors, or neuropeptides. A wide variety of pharmacological effects are mediated by these peptides.

The present invention provides direct means to isolate a group of related receptors displaying both distinctness and similarities in structure, expression, and function. Elucidation of many of the physiological effects of

the bombesin-like peptides will be greatly accelerated by the isolation and characterization of distinct members of the receptor family. In particular, the present invention provides useful probes for identifying additional homologous proteins, as described in Example 29. The human R3BP is one such example. These additional proteins are candidates for receptors which bind other bombesin-like peptides, e.g., phyllolitorin or litorin.

The isolated genes will allow transformation of cells lacking expression of related receptors, e.g., either specie types or cells which lack corresponding receptors and exhibit negative background activity. Expression of transformed genes will allow isolation of pharmacologically pure cell lines, with defined or single receptor subtypes. This approach will allow for more sensitive detection and discrimination of the physiological effects of each receptor subtype in isolation from others. Subcellular fragments, e.g., cytoplasts or membrane fragments, can be isolated and used.

Although the various receptors often have unrelated functions, they share significant structural similarities. Dissection of its structural elements which effect the various physiological functions provided by the receptors is possible using standard techniques of modern molecular biology, particularly in comparing members of a related class. See, e.g., the homolog-scanning mutagenesis technique described in Cunningham et al. (1989) Science 243:1339-1336; and approaches used in O'Dowd et al. (1988) J. Biol. Chem. 263:15985-15992; and Lechleiter et al. (1990) EMBO J. 9:4381-4390; each of which is incorporated herein by reference.

In particular, ligand binding segments can be substituted between receptors to determine what structural features are important in both ligand binding affinity and specificity. The segments of receptor accessible to an extracellular ligand would be primary targets of such analysis. An array of different receptors will be used to screen for ligands exhibiting combined properties of interaction with different receptor subtypes. Particularly interesting segments

of those receptors include, without limitation, the third transmembrane segment, the amino end of the cytoplasmic segment, the second cytoplasmic loop, and the cysteine residues in the cytoplasmic COOH-tail.

Intracellular functions would probably involve segments of the receptor which are normally accessible to the cytosol. However, receptor internalization may occur under certain circumstances, and interaction between intracellular components and the designated "extracellular" segments may occur. These intracellular functions usually involve signal transduction from ligand binding; and G-protein interaction has been reported. The specific segments of interaction of receptor with G-protein may be identified by mutagenesis or direct biochemical means, e.g., cross-linking or affinity methods. Structural analysis by crystallographic or other physical methods will also be applicable. Identification of the similarities and differences between receptor subtypes exhibiting distinct functions will lead to new diagnostic and therapeutic reagents or treatments.

Further study of the expression and control of these receptor subtypes will be useful. The controlling elements associated with the receptors exhibit differential developmental tissue specific, or other expression patterns. Upstream or downstream genetic regions, e.g., control elements, are of interest.

Structural studies of the receptor subtypes will lead to design of new ligands, particularly analogues exhibiting agonist or antagonist properties. This can be combined with previously described screening methods to isolate ligands exhibiting desired spectra of activities.

Expression in other cell types will often result in glycosylation differences in a particular receptor. Various receptor subtypes may exhibit distinct functions based upon structural differences other than amino acid sequence. Differential modifications may be responsible for differential function, and elucidation of the effects are now made possible.

Thus, the present invention provides many receptors for bombesin-like peptides, and reagents developed from them. Although the foregoing description has focused primarily upon the GRP receptor, those of skill in the art will immediately recognize that the invention encompasses receptors for other bombesin-like peptides, e.g. a NMB receptor and an R3BP.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions in any manner.

EXPERIMENTAL

EXAMPLE 1

Preparation of Mouse 3T3 Fibroblast Membranes

Mouse Swiss 3T3 fibroblasts were grown to confluence in Dulbecco's modified Eagles medium supplemented with 10% (vol/vol) fetal calf serum in T-850 roller bottles (lots of 100) at 37° C in a 10% CO_2/90% air environment. Upon harvest, the medium was poured off and each bottle was rinsed twice with 50 ml calcium/magnesium free phosphate buffered saline (PBS-CMF). Cells were incubated with 25-30 ml 0.04% (wt/vol) EDTA in PBS-CMF (warmed to 37° C) for 15 minutes at room temperature. The cells were then removed with firm knocks and pipetted into conical 250 ml centrifuge tubes on ice. Cells from six roller bottles were combined into each centrifuge tube. Roller bottles were rinsed a final time with 25 ml PBS-CMF. Cells were pelleted at 1800 rpm for 10 minutes at 4° C in a Sorvall RC-3B centrifuge. Each pellet was resuspended in 50 ml fresh PBS-CMF at 4° C. Cells from 2-3 centrifuge tubes were combined, pelleted and washed with an additional 120 ml PBS-CMF. The final cell pellets were resuspended in 200 ml lysis buffer (50 mM HEPES, pH 7.5, 2 mM $MgCl_2$, 1 mM EGTA, 50 μg/ml leupeptin, 2.5 μg/ml pepstatin, 10 μg/ml aprotinin, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF)). Cells were lysed by N_2 cavitation. Briefly, 100 ml of the cell suspension was placed in ice in a sealed stainless steel container which was pressurized to 900 psi of N_2. The suspension was slowly released from the chamber through a small orifice into a collection tube, causing rapid decompression and lysis of the cells. Cell lysis appeared complete by microscopic visualization. Membranes were pelleted at 39,000 x g for 30 minutes at 4° C, resuspended in lysis buffer and pelleted again. The pellet was suspended at a concentration of 15 mg membrane protein/ml in a storage buffer (50 mM HEPES, pH 7.5, 1 mM EGTA, 0.25 M sucrose, 50 μg/ml leupeptin, 2.5 μg/ml pepstatin, 10 μg/ml aprotinin, and 0.5 mM PMSF). Membranes

were aliquoted in volumes of 1 and 5 ml, flash-frozen in liquid N_2, and stored at -80° C.

EXAMPLE 2

Comparison of Detergents for Solubilization of the GRP Receptor

Several detergents employed for receptor extraction in other systems were tested to measure their ability to solubilize GRP receptor from Swiss 3T3 fibroblast membranes. Digitonin, Triton X-100, CHAPS, and CHAPS with CHS were all used to extract membranes at a detergent concentration of 0.50% and all were effective in solubilizing receptor that had been radio-labeled by cross-linking to ^{125}I-GRP. The binding of ^{125}I-GRP (0.02 nM), measured as counts/minute (CPM) bound, was assayed in the presence of the detergent (0.1%) used in the extraction and several concentrations of the unlabeled 14-27 C-terminal amino acids of GRP (GRP14-27), as is shown in Figure 1. Only extraction with CHAPS plus CHS yielded detectable binding activity. Since all detergents were effective in solubilizing the GRP receptor, the failure to observe binding activity in extracts prepared individually with digitonin, Triton X-100, or CHAPS was a result of receptor inactivation during the solubilization process. It was noted however, that partial reactivation of the receptor extracted with CHAPS (without CHS) could be achieved by subsequent addition of CHS. This established that CHS acts as a stabilizer in promoting the active GRP receptor.

Comparison of Detergent Concentration for Solubilization of the GRP Receptor

Swiss 3T3 fibroblast membranes, prepared as in Example 1, were incubated with various concentrations of the detergent CHAPS. After separation of insoluble material by centrifugation, soluble GRP binding activity was measured in the supernatant. When 0.75% (w/v) CHAPS was used to solubilize the GRP receptor, maximal receptor binding was observed, as is shown in Figure 2. However, to obtain maximal solubilization of protein a CHAPS concentration of 1.0% (w/v) or greater was used. The GRP receptor binding declined steadily at higher

detergent concentrations. In order to observe specific GRP binding to receptors solubilized by CHAPS, it was useful to include the stabilizing agent CHS. The ratio of CHAPS:CHS was maintained at 10:1 under both extraction and assay conditions.

Comparison of Stabilizing Agent Concentration for Solubilization of the GRP Receptor

Swiss 3T3 fibroblast membranes, prepared as in Example 1, were solubilized with 0.75% (w/v) CHAPS in the presence of various amounts of cholesteryl hemisuccinate (CHS). After the removal of insoluble material by centrifugation, soluble GRP receptor binding activity was measured in the supernatant at a 0.075% (w/v) CHAPS concentration and a CHS concentration 10 fold less than that used in the solubilization step. As shown in Figure 3, the optimal ratio of CHAPS to CHS was about 10:1.

Comparison of Detergent Concentration for Binding Activity of the Solubilized GRP Receptor

The dependency of binding activity on the concentration of detergent was studied. As is shown in Figure 4, GRP binding to the receptor has a narrow optimum between 0.075 and 0.1% CHAPS, and specific binding falls dramatically at CHAPS concentrations greater than 0.4%. Detergent levels above a concentration of 0.4% also cause a large increase in the nonspecific background in the assay which is possibly due to the formation of detergent aggregates. While the GRP receptor is maximally extracted from membranes with detergent levels that are highly inhibitory (0.75%), the inactivation of receptor molecules by CHAPS appeared to be reversible. Complete binding activity of the receptor incubated in 0.75% CHAPS and 0.15% CHS could be recovered upon reducing the concentration of detergent by dialysis.

Optimum pH for GRP Binding

^{125}I-GRP binding was determined in 500 µl of 20 mM MES, 20 mM CHES, 20 mM HEPES, 2 mM EDTA, 10 mg/ml BSA, 30 µg/ml bacitracin, 0.02 nM ^{125}I-GRP, and 5 µg CHAPS extracted membrane protein at several pH values, ranging from pH 5-10. After incubation at 15° C for 30 minutes, samples were cooled on ice. This was followed by the addition of 5.0 ml of 50 mM HEPES, pH 7.5, to neutralize the pH before the separation of bound and

free ligand. Receptor binding was found to be optimal at a pH of 7.5. However, the receptor was able to tolerate incubation at a pH of 10 for at least 24 hours at 4° C without loss of activity. In contrast, incubation of the receptor with a pH 5 buffer at 4° C caused a rapid loss of binding activity.

EXAMPLE 3

Solubilization of the GRP Receptor for Assays

Swiss 3T3 fibroblast membranes, prepared in Example 1, were suspended at 15 mg protein/ml in 50 mM HEPES, pH 7.5, 1.0 mM EGTA, 100 mM NaCl, 0.25 M sucrose, 50 μg/ml leupeptin, 5 μg/ml pepstatin, 10 μg/ml aprotinin, 30 μg/ml bacitracin, and 0.5 mM phenylmethylsulfonyl fluoride. A mixture of 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (CHAPS) and cholesteryl hemisuccinate (CHS) in a ratio of 10:1 was added slowly to yield a final concentration of 0.75% CHAPS. The extract was incubated at 21° C for 30 minutes, cooled to 4° C and the insoluble material was removed by centrifugation at 100,000 x gravity for 60 minutes. The clear supernatant was frozen in liquid N_2 and stored at -80° C without loss of activity.

EXAMPLE 4

Ligand Binding Assays

Specific ^{125}I-GRP (3-(^{125}Iodotyrosyl-15) gastrin releasing peptide, 1900-2000 Ci/mmol) binding to intact or detergent solubilized membranes (20-50 μg, prepared as in Example 3) was assayed in 50 mM HEPES, pH 7.5, 2 mM EDTA, 10 mg/ml bovine serum albumin (BSA), 30 μg/ml bacitracin, and 0.02 nM ^{125}I-GRP. For assays of detergent solubilized membrane extracts, the final CHAPS detergent concentration was adjusted to between 0.050% and 0.20%. The concentration of CHS was maintained at 1/5 to 1/10 the concentration of CHAPS. Samples were also prepared omitting the BSA. After incubation at 15° C for 30 minutes, samples were cooled to 0° C. Bound ligand (^{125}I-GRP:GRP receptor complex) was recovered by rapid filtration through polyethyleneimine treated Whatman GF/B

filters, followed by four washes with 4 ml of ice cold TRIS buffer (50 mM TRIS/Cl, pH 7.5). The filters were counted in an Isodata 500 gamma counter. Nonspecific backgrounds were determined by inclusion of 100 nM unlabeled GRP in the assay to compete for specific binding sites and typically represented 1.5-2% of the specific radioactivity bound. The nonspecific binding could be attributed to a small degree of binding of the ^{125}I-GRP to the filters. It was found that binding activity of the solubilized receptor is highly dependent on the concentration of the detergent. As shown in Figure 4, GRP binding to the receptor has a narrow optimum between 0.075% CHAPS/0.015% CHS and 0.10% CHAPS/0.02% CHS, and specific binding falls dramatically at CHAPS/CHS concentrations greater than 0.4%/0.08%. Detergent levels above about 0.4% CHAPS with 0.08% CHS present also cause a large increase in the nonspecific background possibly due to the formation of detergent aggregates. Since the receptor is maximally extracted from membranes with detergent levels that are highly inhibitory (0.75% CHAPS), inactivation of the receptor by CHAPS appeared to be reversible. Indeed, complete binding activity of receptor incubated in 0.75% CHAPS plus 0.15% CHS could be recovered upon reducing the concentration of detergent by dialysis.

EXAMPLE 5

Receptor Kinetics

Assays were performed for various times of incubation and BSA (10 mg/ml) was either included in the assay or omitted. ^{125}I-GRP binding to the soluble receptor at 15° C leveled off by 20 minutes and remained constant for up to 2 hours. Omission of the BSA that had been added to prevent proteolysis of the ligand had no significant effect on the binding kinetics.

EXAMPLE 6

G-Protein Complex

The GRP receptor in Swiss 3T3 fibroblast membranes was found to be G-protein coupled. Therefore, the effect of guanylnucleotides on ^{125}I-GRP binding to soluble receptors was studied. The final detergent concentration was 0.075% CHAPS and 0.015% CHS was present. The G-protein coupling of the GRP receptor in intact Swiss 3T3 fibroblast membranes was inferred from the observation that the ligand affinity of the receptor was reduced about ten fold in the presence of the nucleotides GDP and GTP and the non-hydrolyzable GTP analogue GMPPNP. In the presence of Mg^{+2}, guanylnucleotides are presumed to induce the dissociation of G-proteins from the high affinity ligand/receptor/G-protein ternary complex, resulting in formation of the ligand/receptor complex that displays lower affinity. The GRP receptor extracted from membranes by CHAPS showed no change in their ligand binding properties in the presence of Mg^{+2} and GTP or GMPPNP at levels that reduce GRP binding to membranes by about 80%. The lack of an effect of GTP on GRP binding in the presence of Mg^{+2} indicates that interaction of the receptor with its G-protein is not maintained in the detergent extract. The control in Table 5, contains $MgCl_2$.

TABLE 5: GRP binding in presence of guanylnucleotide

	Solubilized Membranes Counts/minute Bound ^{125}I-GRP Measured as % of Total Added
control	28
control + 10 mM AMPPNP	27.8
control + 10 mM GTP	27.5
control + 10 mM GMPPNP	26.5
control + 10 mM GMPPNP + 100 nM GRP1-27	2.0

	Intact Membranes Counts/minute Bound ^{125}I-GRP Measured as % of Total Added
control	28.9
control + 5 mM ATP	29.7
control + 5 mM AMPPNP	33.4
control + 5 mM GTP	10.7
control + 5 mM GMPPNP	10.5
control + 5 mM GMPPNP + 100 nM GRP1-27	1.4

EXAMPLE 7

Scatchard Analysis of the Soluble GRP Receptors

Scatchard analysis of ^{125}I-GRP binding to intact and solubilized Swiss 3T3 membranes was done. One particular experiment is discussed below, where the binding parameters of the solubilized and the membrane bound form of the receptor are determined under similar conditions. Assays were determined at 15° C. For assays of solubilized or intact membranes, the binding reactions were terminated at 30 and 180 minutes, respectively. The following are the binding parameters, where K_D is the dissociation constant and Bm is the maximum binding capacity:

K_D (intact membranes)	= 37 pM
K_D (solubilized membranes)	= 10 pM
Bm (intact membranes)	= 0.79 pmol/mg protein
Bm (solubilized membranes)	= 1.0 pmol/mg protein

Scatchard analysis revealed the presence of a high affinity binding site. Some non-linearity and scatter in the data was observed at low values of bound/free ligand where determination of precise binding data is most difficult. The dissociation constant of the ligand binding to the soluble receptors (10 pM) was less than that exhibited by the receptors in intact membranes (37 pM) despite the lack of G-protein coupling to the soluble receptors that was observed. As noted above, such G-protein coupling boosts the affinity of the membrane receptors by an order of magnitude. However, the assay was performed under conditions that had been optimized for GRP binding to the soluble receptor which may have compensated for the affinity lost by G-protein interactions. In other experiments, the dissociation constant of the solubilized receptor was calculated to range from 10 to 30 pM. The data demonstrated that the functional conformation of the receptor binding site was maintained in detergent solution.

The Scatchard data from this experiment also indicated that there were 0.79 pmol receptors/mg protein in crude Swiss 3T3 cell membranes and about 50% of the receptor

binding sites were solubilized by extracting the membranes with detergent. Some of the factors that were found to be necessary for the most efficient solubilization of receptor activity were inclusion of NaCl (>100 mM), elimination of divalent cations, and the extraction of membranes at room temperature. Although NaCl was necessary for the optimal solubilization of the receptors, the salt inhibited GRP binding to both the Swiss 3T3 fibroblast membranes and detergent solubilized receptor (IC_{50} = approx. 50 mM). However, the inhibition of the receptors by NaCl at concentrations up to 1.0 M was found to be completely reversible.

EXAMPLE 8

Ligand Specificity of GRP Binding Sites in Soluble Membrane Extracts

The binding of ^{125}I-GRP to solubilized 3T3 membranes was assayed in the presence of various unlabeled competitor peptides. The C-terminal eight amino acids of GRP (GRP20-27) were found to be essential for high affinity binding to the GRP receptors in whole cells. The complete GRP sequence (GRP1-27), the N-terminal portion of GRP (GRP1-16), substance P, substance P antagonist, physalemin (all of which were from Peninsula Laboratories, Belmont CA), and the C-terminal portion of GRP with norleucine substituted for methionine referred to as [Nle14,27]GRP13-27 ((i.e. Lys-Nle-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Nle-NH_2), were tested for their ability to compete for ^{125}I-GRP binding to soluble 3T3 fibroblast membrane extracts. The concentration of [Nle14,27]GRP13-27 required to cause 50% inhibition of ^{125}I-GRP binding to the soluble receptor (IC_{50} = 0.3 nM) was slightly higher than that of GRP1-27 (IC_{50} = 0.1 nM). In contrast, the N-terminal portion (GRP1-16) was unable to compete with ^{125}I-GRP for binding to the soluble receptor. Additionally, substance P, substance P antagonist, and physalemin had no inhibitory effect at the concentrations tested (up to 1000 nM). These results parallel closely that which was found in similar studies in whole cells and isolated membranes.

EXAMPLE 9

Cross-linking of ^{125}I-GRP Receptors

The molecular weight of the GRP receptor in solubilized Swiss 3T3 membranes was estimated by covalently cross-linking it to bound ^{125}I-GRP via the homobifunctional cross-linking reagent bis(sulfosuccinimidyl)suberate (BS3) and analyzing the affinity of labeled receptor by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). This cross-linker is specific for primary amino groups. Soluble 3T3 fibroblast membrane protein (40 μg) was incubated for 30 minutes at 15° C in a final volume of 500 μl of 50 mM HEPES, 2 mM EDTA, 0.075% CHAPS, 0.015% CHS, 30 μg/ml bacitracin, and 0.2 nM ^{125}I-GRP. The binding reaction was cooled to 0° C and BS^3 was added to yield a final concentration of 3 mM. Cross-linking was quenched by addition of 0.10 ml of TRIS buffer (1.0 M TRIS/Cl, pH 7.5). After another 10 minute incubation, 0.1 ml TCA (100%) was added and the solution was further incubated at 0° C for 30 minutes. Precipitated material was collected by centrifugation, washed with ice cold acetone, and heated at 95° C for 3 minutes in SDS-PAGE sample buffer. The samples were subjected to SDS-PAGE on a 7.5% gel and the gel was fluorographed. A detailed description of the SDS-PAGE technique is found in Laemmli et al. (1970) Nature 227:680, which is incorporated herein by reference. Figure 5 illustrates the gel display.

Lane	Composition
A	no addition
B	0.1 nM unlabeled GRP
C	0.5 nM unlabeled GRP
D	1.0 nM unlabeled GRP
E	100 nM unlabeled GRP

A strongly labeled species migrated in a diffuse band with an apparent Mr of about 75-100 kDa. Low levels of unlabeled GRP inhibited the labeling of this species, indicating that the labeling is highly specific. The broadness of the labeled band is consistent with the fact that the GRP receptor has been

found to contain carbohydrate. The labeled product is very similar to that derived from whole cell or membrane cross-linking experiment. N-Glycanase treatment of samples derived from cross-linked whole cells indicated that the labeled protein contained N-linked carbohydrates. The deglycosylated protein displayed an apparent Mr of 38 kDa on SDS-PAGE.

EXAMPLE 10

Purification of the GRP Receptor

Solubilization of the GRP Receptor

Swiss 3T3 fibroblast membranes (2-3 g of protein) were prepared as described in Example 1 and suspended in 200 ml storage buffer (see Example 1). The membranes were mixed with 50 ml of NaCl (5.0 M), bringing the NaCl concentration to about 1 M, pelleted by centrifugation at 40,000 x g for 30 minutes, and washed twice at 4° C with 200 ml of high salt buffer (50 mM HEPES, pH 7.5, 2 mM EDTA, 1.0 M NaCl, 25 μg/ml leupeptin, 10 μg/ml aprotinin, 2.5 μg/ml pepstatin, and 0.5 mM PMSF). The membranes were then washed with low salt buffer (50 mM HEPES, pH 7.5, 2 mM EDTA, 25 μg/ml leupeptin, 10 μg/ml aprotinin, 2.5 μg/ml pepstatin, and 0.5 mM PMSF) and resuspended in 200 ml 50 mM HEPES, pH 7.5, 2 mM EDTA, 1 mM EGTA, 100 mM NaCl, 0.03 μg/ml bacitracin, 25 μg/ml leupeptin, 10 μg/ml aprotinin, 2.5 μg/ml pepstatin, and 0.5 mM PMSF. A stock solution containing a mixture of CHAPS and CHS was added slowly to the membranes to give a final concentration of 0.75% CHAPS and 0.075% CHS. The mixture was incubated for 30 minutes at 21° C, cooled to 4° C and centrifuged at 100,000 x g or 60 minutes at 4° C. The supernatant contained the solubilized GRP receptor.

Precipitation by Polyethylene Glycol

To the solubilized extract (190 ml), 126 ml of ice cold polyethylene glycol (PEG) 8,000 (50 w/v% in H_2O) was added. After thorough mixing, the precipitate that formed was collected by centrifugation at 100,000 x g for 10 minutes. The pellet was suspended in 25 mM HEPES, 25 mM TRIS, pH 7.5, 2 mM EDTA, 0.075% CHAPS, 0.0075% CHS, 5 μg/ml leupeptin, and 10

μg/ml bacitracin in a total volume of 50 ml with the aid of a Potter-Elvehjem homogenizer. The suspension, which contained some insoluble protein, was centrifuged at 69,000 x g for 10 minutes, and the pellet was discarded.

Wheat Germ Agglutinin Chromatography

Following precipitation by PEG, the GRP receptor was further purified by lectin affinity chromatography. A column (1.6 x 9 cm) containing wheat germ agglutinin-agarose resin (3-5 mg lectin/mg of wet gel) (E-Y Laboratories, San Mateo, CA) was equilibrated with 50 mM HEPES, pH 7.5, 2 mM EDTA, 0.25% CHAPS, 0.025% CHS, 5 μg leupeptin, and 10 μg/ml bacitracin at 4° C. The soluble extract was diluted with one volume of column buffer, and the final detergent concentration was adjusted to 0.25% CHAPS and 0.025% CHS. The sample was applied to the lectin column at a flow rate of 1.5 ml/min. The column was then washed with about 10 column volumes of buffer, and eluted with column buffer plus 5 mM N,N',N''-triacetyl-chitotriose. Fractions containing the GRP receptor binding activity were pooled and diluted with 2.3 volumes of 25 mM HEPES, 25 mM TRIS, pH 7.5, 2.0 mM EDTA, 5 μg/ml leupeptin, and 10 μg/ml bacitracin.

GRP Affinity Chromatography

Actigel superflow resin (10 ml)(Sterogene, San Gabriel, CA) was washed with 5 volumes of 100 mM KPO_4, pH 7.0. The resin was incubated with 10 ml of 100 mM KPO_4, 100 mM $NaCNBH_3$, pH 7.0 containing 2 mg/ml [Nle14,27]GRP13-27 for 2 hours with gentle agitation. The resin was washed with 100 mM KPO_4, pH 7.0, followed by alternating washes with 100 mM KAc, pH 4.0, 0.5 M NaCl; and 100 mM TRIS pH 8.0, 0.5 M NaCl. A column of the resin (1.6 x 5 cm) was equilibrated with 25 mM TRIS, 25 mM HEPES, pH 7.5, 2.0 mM EDTA, 0.075% CHAPS, 0.0075% CHS, 5 μg/ml leupeptin, and 10 μg/ml bacitracin at 4° C. The crude GRP receptor eluted from the lectin column was loaded onto the GRP affinity column at 0.1 ml/min. The column was then washed with about 20 volumes of the equilibration buffer. The bound receptor was eluted from the column with equilibration buffer plus 0.5 M NaCl at a flow rate of 0.2

ml/min. Fractions containing the receptor were identified by assays of ^{125}I-GRP binding activity and were pooled (10-13 ml). The elution pool was concentrated to about 1 ml by ultrafiltration using a Centriprep-10 device (Amicon, Danvers, MA). The sample was then desalted by dilution of the sample with 15 volumes of affinity column equilibration buffer and re-concentration of the sample to 1 ml. This desalting step was repeated and the resulting 1 ml sample was diluted to 5 ml with affinity column equilibration buffer. PAGE analysis of the purified GRP receptor revealed the presence of a significant level of contamination.

This solution of semi-pure receptor was loaded onto a second [Nle14,27]GRP13-27-actigel superflow column (1.0 x 3 cm), prepared as described above, at 1.8 ml/h. The column was washed with 20 column volumes of equilibration buffer, and the bound receptor was eluted with equilibration buffer plus 0.5 M NaCl at a flow rate of 0.1 ml/min. Fractions containing GRP receptor binding activity were pooled and concentrated to 0.3 ml by ultrafiltration.

Gel Filtration

The purified receptor was desalted by chromatography on a Superose-6 HR 10/30 column (Pharmacia LKB, Piscataway, NJ). The column was equilibrated with 20 mM HEPES, pH 7.5, 2 mM EDTA, 0.075% CHAPS, 0.0075% CHS, and 100 mM NaCl. The receptor was chromatographed at 0.4 ml/min. The receptor was eluted from the column in about 2 ml.

Characterization of the Purified GRP Receptor

The overall yield of the pure GRP receptor from the crude solubilized extract ranged from 10-20%, based on recovery of high affinity ^{125}I-GRP binding activity. Scatchard analysis of binding data obtained with the purified receptor indicated that its affinity for GRP (K_D = 10-30 pM) was essentially the same as the receptor in the crude detergent solubilized extract. The data show that 30-50 pmoles of receptor sites are typically obtained in the final purified fractions of the receptor, as outlined in this example. This corresponds to about 1-2 μg of receptor protein, taking into account that the

deglycosylated receptor exhibits an apparent molecular weight of 36±5 kilodaltons on SDS-PAGE gels.

A silver stained SDS-PAGE gel of the receptor preparation showed a single intensely staining diffuse band with an apparent molecular weight of 70-100 kD. The receptor preparation was essentially free of contaminants. Figure 6 illustrates the silver stained gel display of the purified GRP receptor. The relative level of silver staining of the GRP receptor band was compared with known amounts of protein to determine the approximate amount of receptor protein loaded on the gel. The rough value obtained was in the range of that estimated to be present by Scatchard analysis of ^{125}I-GRP binding data, which confirmed that the intensely staining band on the gel was the GRP receptor. Furthermore, the apparent molecular weight of the purified GRP receptor corresponded to that obtained with affinity labeled receptor. This was obtained by binding ^{125}I-GRP to the receptor in whole cells, intact membranes, or crude soluble extracts, and cross-linking the receptor-ligand complex with a homobifunctional cross-linking reagent.

The diffuse nature of the GRP receptor band on SDS PAGE is characteristic of proteins containing carbohydrate. A small portion of the purified receptor was radiolabeled by iodination with ^{125}I-NaI in the presence of Iodogen (Pierce, Rockford, IL) to enhance the detection of the receptor on gels. Treatment of the radiolabeled receptor with N-glycanase resulted in loss of the 70-100 kDa band, and the generation of a new band at about 36±5 kilodaltons, representing the deglycosylated receptor.

Determination of Partial Amino Acid Sequence of the GRP Receptor

A partial sequence near the N-terminus of the purified GRP receptor was determined by sequential Edman degradation. The sequence obtained for residues 8-17 was:

-Leu-Asn-Leu-Asp-Val-Asp-Pro-Phe-Leu-Ser-.

EXAMPLE 11

Trypsinization of the Purified GRP Receptor and the Isolation of Tryptic fragments

Purified GRP receptor was prepared as described in Example 10. After Superose-6 chromatography, 40 picomoles of receptor were obtained based on Scatchard analysis of ^{125}I-GRP binding data. This corresponded to about 1.6 μg of protein. The sample (3 ml) was concentrated to about 100 μl by ultrafiltration using a Centricon 10 device (Amicon). The sample was then diluted with 2 ml of H_2O, and concentrated to 100 μl. Once again, the sample was diluted with 2 ml H_2O, and concentrated to 100 μl, and was finally diluted with 1 ml of H_2O, and concentrated to 138 μl. To digest the receptor with trypsin, 0.1 μg of trypsin was added, and the sample was incubated at 37° C. After 2 hours, an additional 0.1 μg of trypsin was added, followed by another 0.2 μg of trypsin after 5 hours of incubation. After 22 hours at 37° C, the sample was rapidly frozen in liquid N2 and stored at -80° C.

Trypsin digested GRP receptor was thawed to room temperature and reduced with dithiothreitol (DTT) at a final concentration of 10 mM for 30 minutes at 37° C. The entire DTT treated tryptic digest was then fractionated by reverse phase high pressure liquid chromatography (HPLC) using a 2.1 mm X 3 cm C4 column (Brownlee, Aquapore Butyl, 300 angstrom pore size), and a linear gradient of 0.05% trifluoroacetic acid (TFA) in water (solvent A) to 0.05% TFA in 100% acetonitrile (solvent B), see Figure 7. The conditions for the HPLC gradient were 0% solvent B to 100% solvent B in 60 minutes at a flow rate of 0.2 milliliters per minute. Effluent fractions were detected at 215 nm, collected at one minute intervals, and stored at 4° C.

For peptide sequence analysis, consecutive fractions were pooled and concentrated on a Speed Vac (Savant, Farmingdale, NY) to a final volume of approximately 50 μl. The sample was loaded in entirety onto a glass fiber filter which had been treated and precycled with Biobrene (Applied Biosystems (ABI), Foster City, CA). Automated amino acid sequence analysis was performed on an ABI model 475A gas phase

sequencer according to Hewick et al. (1981) J. Biol. Chem. 256:7990-7997, equipped with an ABI model 120A on-line detection HPLC system for identification of phenylthiohydantoin (PTH-) amino acids. Quantitation of PTH-amino acids was performed by an ABI model 900 data system using 60 picomoles of a set of known PTH-amino acid standards (ABI). In this manner, the combined tryptic HPLC fractions 56 through 59 gave the amino acid sequence MASFLVFYVIPLAII (designated T56/59); the tryptic HPLC fraction 44 yielded the amino acid sequence QLTSVGVSV (designated T44), and the tryptic HPLC fraction 50 gave the amino acid sequence PNLFISXLALG (designated T50), where X denotes a residue that could not be identified.

NH_2-terminal sequence analysis was performed on the intact purified GRP receptor following washing of the sample with H_2O and concentration of the sample on a Centricon 10 ultrafiltration device (Amicon, Danvers, MA). The sample (95% or approximately 95 μl was loaded onto a Biobrene (ABI) precycled glass filter and NH_2-terminal sequence analysis was performed through 30 cycles of automated Edman degradation on an ABI 475A gas phase sequencer (Hewick et al.(1981)). PTH-amino acid identification and quantitation were performed using an ABI 120A PTH-amino acid analyzer and ABI 900 data system. Following two separate NH_2-terminal sequence runs on two purified preparations of the GRP receptor, the following consensus NH_2-terminal amino acid sequence was obtained for 17 residues, where X denotes a residue for which an accurate assignment of a specific amino acid was not made:

1				5				10				15			
A	P	N	X	X	S	X	L	N	L	D	V	D	P	F	L	S.

EXAMPLE 12

Identification of cDNA Clone Encoding the Swiss 3T3 GRP Receptor

Preliminary studies established that a murine embryonal fibroblast cell line (Balb 3T3) expressed a repertoire of mRNAs very similar in abundance and distribution to the GRP receptor-expressing Swiss 3T3 murine fibroblast cell line, but did not have any cell surface GRP receptors

detectable in standard binding assays See Kris et al. (1987) J. Biol. Chem. 262:11215-11220; and Zachary et al. (1985) Proc. Natl. Acad. Sci. USA 82:7616-7620, each of which is incorporated herein by reference. These observations suggested that the GRP receptor mRNA would be one of a limited number of transcripts present in Swiss 3T3, but absent from Balb 3T3 mRNA. Polyadenylated mRNA was isolated from both Swiss 3T3 and Balb 3T3 cell lines and was used to generate a Swiss 3T3 subtracted cDNA library enriched for cDNAs derived from Swiss 3T3 mRNA but not represented in Balb 3T3 mRNA using published methodology, e.g., Timlin et al. (1990) Nuc. Acids. Res. 18:1587-1593, which is incorporated herein by reference. The cDNA inserts whose length exceeded 300 base pairs were ligated into the lambda gt10 bacteriophage cDNA cloning vector and the library amplified using the established methods, e.g., Davis et al. (1986) Basic Methods in Molecular Biology, Elsevier Science Publishing Company, New York.

The library was screened with an oligonucleotide probe whose sequence was based on the amino acid sequence of an internal tryptic fragment (T 56/59) purified by HPLC from a tryptic digest of the purified GRP receptor protein. The amino acid sequence (MASFLVFYVIPLAII) of the internal peptide was used to design a long non-degenerate antisense oligonucleotide whose sequence was based on optimal codon usage frequency as described in the literature by Lathe (1985) Mol.Biol. 183:1-12, resulting in a 44-base long probe referred to as I3: (5'ATGATGGCCAGGGGGATCACATAGAAGACCAGGAAGGAGGCCAT 3'). The I3 probe was labeled by phosphorylation of the 5' end using gamma ^{32}p-ATP and polynucleotide kinase employing the established techniques of Davis et al. (1986). The labeled probe was used to screen 100,000 member clones from the subtracted library using hybridization and wash conditions as described. See Wood (1987) Chapter 48 in Methods in Enzymology 152:443-447, which is incorporated herein by reference. Duplicate screening identified five positive clones, which were plaque purified. The EcoRI inserts from the five clones were subcloned into the plasmid vector pGEM 4 (Promega), and the nucleotide sequence of

the hybridizing inserts was determined using the Sequenase 2.0 double stranded sequencing kit (US Biochemical). Two of the five clones (T1 and T2) had an identical region of overlapping DNA sequence which encoded the internal peptide used to design the oligonucleotide probe. The fragment was removed from the plasmid vector by EcoRI digestion and purified by gel electrophoresis and electroelution as described by Davis et al. (1986). The purified insert fragments were labeled by random primer extension using a commercially available kit and the supplier's recommendations (Bethesda Research Laboratories) to generate a probe to identify other overlapping cDNA clones from the subtracted library in a second screening of the 100,000 library members. Nucleotide sequence analysis of the nine additional clones identified revealed a single long open reading frame whose predicted translation product included the internal tryptic fragment amino acid sequence, which ended in a termination codon within the composite sequence. The amino terminal end of the open reading frame was not present in any of the clones isolated from the subtracted library.

To obtain the 5' end of the cDNA and the sequence at the amino terminal end of the open reading frame, an in vitro polymerase chain reaction amplification (PCR) cDNA cloning procedure (5' RACE) was performed essentially as described in Frohman et al. (1988) Proc. Natl. Acad. Sci. USA 85:8998-9002, using two nested gene-specific oligonucleotides (EXT 3: 5' GGGGAGCCAGCAGAAGGC 3'; EXT 2: 5' CCATGGAATGGATTTTA) derived from the known nucleotide sequence of the cDNA clones previously analyzed. EXT 3 was used as a gene-specific primer for reverse transcription of Swiss 3T3 mRNA, and EXT 4 was used as a gene specific primer for Taq DNA polymerase catalyzed PCR. Nineteen 5' RACE cDNAs were isolated and characterized, and five of the clones that extended the longest distance were sequenced as described previously. Nucleotide sequence analysis revealed an extension of the long open reading frame encoding the internal tryptic peptide amino acid sequence, beginning with an initiator methionine codon. The predicted amino acid sequence of the open reading frame was compared with

amino terminal sequence derived from the purified GRP receptor (See Example 11). The experimentally determined amino acid sequence did not contain the methionine at position 1 of the deduced sequence, but corresponded well to residues 2-18. Deduced amino acids 2-4 and 8-18 (Table 1) were identical. The amino acids that did not match (amino acids 5-7, Table 1) were ambiguous in the original amino acid sequence, probably because they are located at an N-linked glycosylation site (Asn-Cys-Ser). In addition, the amino acid sequence from internal tryptic peptides T44 (QLTSVGVSV) and T50 (PNLFISXLALG), derived from the purified Swiss 3T3 GRP receptor (Example 11), matched segments within the long open reading frame of the composite GRP receptor cDNA.

Gene-specific primer-directed cDNA cloning was used to obtain a single cDNA clone which encodes the entire uninterrupted open reading frame. In this procedure, a gene-specific oligonucleotide (EXT7: 5' TACTTTGAGATACAATGG 3') complementary to an 18 nucleotide segment of the 3' untranslated region of the GRP receptor mRNA was used to prime the synthesis of first-strand cDNA by MuLV reverse transcriptase. Double-stranded cDNA was generated, and cloned into lambda gt10 using standard methodology of Davis et al. (1986). Five hundred thousand clones were screened with a cDNA fragment probe derived from one of the 5' RACE cDNA clones which extended into the 5' untranslated region of the cDNA. Over twenty clones were identified, and ten were plaque purified and subcloned into plasmid vectors by standard methods of Davis et al. (1986). Nucleotide sequence analysis confirmed that the clones contained the entire uninterrupted open reading frame of the GRP receptor protein. The DNA sequence of the GRP receptor from mouse and its deduced amino acid sequence is shown in Table 1.

Analysis of the nucleotide sequence of the open reading frame revealed several interesting features of the predicted protein. The predicted molecular weight of the protein is about 43,100 daltons, in good agreement with that reported for the N-glycanase treated GRP binding protein from

Swiss 3T3 cells, described in Example 10. Hydrophobicity analysis is presented in Figure 8 and predicts the presence of seven putative transmembrane domains, consistent with earlier observations that the GRP receptor is coupled to a guanine-nucleotide binding protein (G-protein), see Fischer et al. (1988) J. Biol. Chem. 263:2808-2816. The superfamily of G-protein coupled receptor genes typically share certain conserved residues within or adjacent to the seven transmembrane domains, see Masu et al. (1987) Nature 329:836-838. These conserved amino acids are found in the predicted locations within the open reading frame of the mouse GRP receptor sequence (Table 1). Five potential sites for N-linked glycosylation (Asn-X-Ser/Thr) are noted (Table 1), consistent with the observation that the GRP receptor is heavily glycosylated, and that N-glycanase treatment of the GRP receptor glycoprotein reduces the apparent molecular weight of the protein in SDS- polyacrylamide gels from about 70-100 kilodaltons to about 38±5 kilodaltons (Example 10). Table 6 shows a comparison between the GRP receptor and the substance K receptor.

Table 6: A comparison of the amino acid sequences of the GRP receptor and the Substance K receptor.

```
  1 MAPNNCSHLNLDVDPFLSCNDTFNQSLSPPKMDNWFHPGFIYVIPAVYGL  50 ■
  1 .MGACVVMTDINISSGLDSNATGITAFSMPGWQ.......LALWTAAYLA  42 ▲
 51 IIVIGLIGNITLIKIFCTVKSMRNVPNLFISSLALGDLLLLVTCAPVDAS 100
 43 LVLVAVMGNATVIWIILAHQRMRTVTNYFIVNLALADLCMAAFNAAFNFV  92
101 KYLADRWLFGRIGCKLIPFIQLTSVGVSVFTLTALSADRYKAIVRPMDIQ 150
 93 YASHNIWYFGRAFCYFQNLFPITAMFVSIYSMTAIAADRYMAIVHPFQPR 142
151 ASHALMKICLKAALIWIVSMLLAIPEAVFSDLHPFHVKDTNQTFISCAPY 200
143 LSAPGTRAVI..AGIWLVALALAFPQCFYSTI....TTDEGATKCVVAWP 186
201 PHSNELHPKIHSMASFLVFYVIPLAIISVYYYFIARNLIQSAYNLPVEGN 250
187 EDSGGKMLLLYHLIVIALIYFLPLVVMFVAYSVIGLTLWRRSVPGHQAHG 236
251 IHVKKQIESRKRLAKTVLVFVGLFAFCWLPNHVIYLYRSYHYSEVDTSML 300
237 ANL.RHLQAKKKFVKTMVLVVVTFAICWLPYHLYFILGTF...QEDIYCH 282
301 HFVTSICAHLLAFTNSCV..NPFALYLLSKSFRKQFNTQLLCCQ...... 342
283 KFIQQVYLALFWLAMSSTMYNPIIYCCLNHRFRSGFRLAFRCCPWVTPTE 332
343 .........PGLMNRSHSTGRSTTCMTSFKSTNPSATFSLINRNICHEGY 383
333 EDKMELTYTPSL...STRVNRCHTKEIFFMSGDVAPSEAVNGQAESPQAG 379
384 V*.... 385
380 VSTEP* 385
```

TABLE 6

LEGEND:
■ GRP RECEPTOR
▲ SUBSTANCE K RECEPTOR

Northern blot analysis was undertaken to identify the nature of the transcripts encoding the Swiss 3T3 GRP receptor. The results are shown in Figure 9. One microgram of polyadenylated mRNA derived from Swiss 3T3 and Balb 3T3 cells was purified and resolved by electrophoresis on a formaldehyde-containing one percent agarose gel, which was subsequently transferred to a nitrocellulose filter. The filter was hybridized with a 450-base pair cDNA fragment probe encoding the carboxy terminal transmembrane domains 5, 6, and 7 as well as a portion of the 3' untranslated sequences. The probe was labeled with ^{32}P to a specific activity 500 cpm/picogram using a commercially available random primer extension kit (Bethesda Research Laboratories). Two mRNAs specifically hybridized to the probe, whose sizes were estimated to be 7.2 kb and 3.0 kb by comparison to mouse 28S (5.0 kb) and 18S (2.0 kb) markers (Figure 9). As expected, the two mRNA forms were only detected in mRNA from Swiss 3T3, with no GRP receptor transcripts observed in mRNA from Balb 3T3 cells.

EXAMPLE 13

Human mRNA Species Homologous to Mouse GRP Receptor cDNA

Northern blot analysis was performed to determine the degree of homology between the GRP receptor expressed in human fetal lung cells, see Kris et al. (1987) J. Biol. Chem. 262:11215-11220; and the Swiss 3T3 cell receptor. Polyadenylated mRNA was isolated from human fetal lung cells, and subjected to Northern analysis as described in Example 12, using the same 450-base pair cDNA fragment of the Swiss 3T3 cell GRP receptor as a probe, except that the stringency of the hybridization filter washing steps was reduced. Two mRNA species of approximately 7.2 and 3.0 kb were detected in the human cell line, corresponding to those observed in mouse Swiss 3T3 cell mRNA. See Figure 10. Based on the conditions used for the blot, the mRNA species identified were at least 80% homologous to the Swiss 3T3 GRP receptor probe. The results indicate that the mouse GRP receptor cDNA, described in Example

12, can be used to readily isolate cDNAs or genomic DNA fragments encoding the GRP receptor in other mammalian species, including humans.

These homologous receptors will be available to isolate other homologous receptors by using similar techniques.

EXAMPLE 14

Expression of the Mouse GRP Receptor Derived from the cDNA Clone in Xenopus Oocytes to Demonstrate Receptor Function

A sense in vitro transcript was prepared from the mouse GRP receptor cDNA protein coding region (Table 1) cloned in the transcription vector pGEM 4 (Promega) using sp6 RNA polymerase and established methods of Davis et al. (1986). The synthesized transcript (about 20 nanograms) was injected into Xenopus oocytes. Sixteen hours later, the oocytes were voltage clamped and bathed in a solution containing 10^{-9} M GRP. As shown in Figure 11, a GRP ligand dependent chloride current (magnitude of about 160 nanoamperes) was coincident with addition of the ligand. These results demonstrate the expression of an in vitro transcript-dependent GRP receptor on the Xenopus oocyte cell surface, which is coupled through G-proteins to a Ca^{++} dependent chloride channel. The ligand dependent chloride current was not observed in control oocytes injected with an antisense in vitro transcript, thus demonstrating specificity of the response.

EXAMPLE 15

Isolation of Candidate NMB-R cDNA Clones

A hexamer-primed cDNA library was constructed from rat esophagus, and screened by hybridization at low stringency with the Swiss 3T3 GRP-R cDNA probe. Several candidate clones were isolated, two of which contained the entire coding region of a long open reading frame. Several criteria were used to establish that the cDNA clones encode a NMB-preferring bombesin receptor protein distinct from the GRP-R initially isolated. The properties distinguishing these two bombesin receptor subtypes include protein structure, sensitivity of receptor

function to specific antagonists, relative binding affinity for bombesin peptide ligands, and tissue distribution of expression. These properties were studied using the cDNA clones isolated at low stringency from the esophageal cDNA library.

EXAMPLE 16

The Nucleotide Sequence and Amino Acid Sequence of NMB-R cDNA

The nucleotide sequence and predicted amino acid sequence of a single long open reading frame present in two independent clones encoding the putative NMB-R is shown in Table 3. These cDNAs derive from mRNAs that encode a protein 390 amino acid in length, with a calculated molecular weight of 43 kDa. A hydropathy analysis of the predicted NMB-R protein reveals seven stretches of hydrophobic amino acids, consistent with a seven transmembrane-structure typical of G-protein coupled receptors. See Figure 12. There are three potential sites for N-linked glycosylation (Asn^{1}, Asn^{71}, Asn^{192}), consistent with the prediction that the NMB-R protein, like the GRP-R, may be a glycoprotein. See Table 3.

In Table 7, the predicted amino acid sequences of the mouse Swiss 3T3 GRP receptor and the rat NMB-R protein are compared. The NMB-R amino acid sequence has higher similarity to the GRP-R than any other sequence reported to date (54% identity). A previously reported comparison of the rat substance P and substance K receptors shows comparable amino acid sequence identity between these two tachykinin receptor subtypes (48% identity), see Yokota et al. (1989) J. Biol. Chem. 264, 17649-17652. In contrast, the sequence identity between the putative rat NMB-R and the mouse GRP-R is considerably lower than observed when the substance K receptors are compared (85%) from bovine, see Masu et al. (1987) Nature 329, 836-838, and rat, see Yokota et al. (1989) J. Biol. Chem. 264, 17649-17652.

Table 7: A comparison of the predicted amino acid sequences of a rat NMB-R with a mouse GRP-R. The predicted amino acid sequence of a rat NMB-R (Table 3) and mouse Swiss 3T3 GRP-R (Table 1) are aligned to maximize homology using the GAP Program in the Software Package of the University of Wisconsin Genetics Computer Group. See Devereaux et al. (1984) Nuc. Acids Res. 12:387-395. Solid lines between amino acid residues which are typically conserved in many other known G-protein coupled receptor superfamily members are enclosed in boxes.

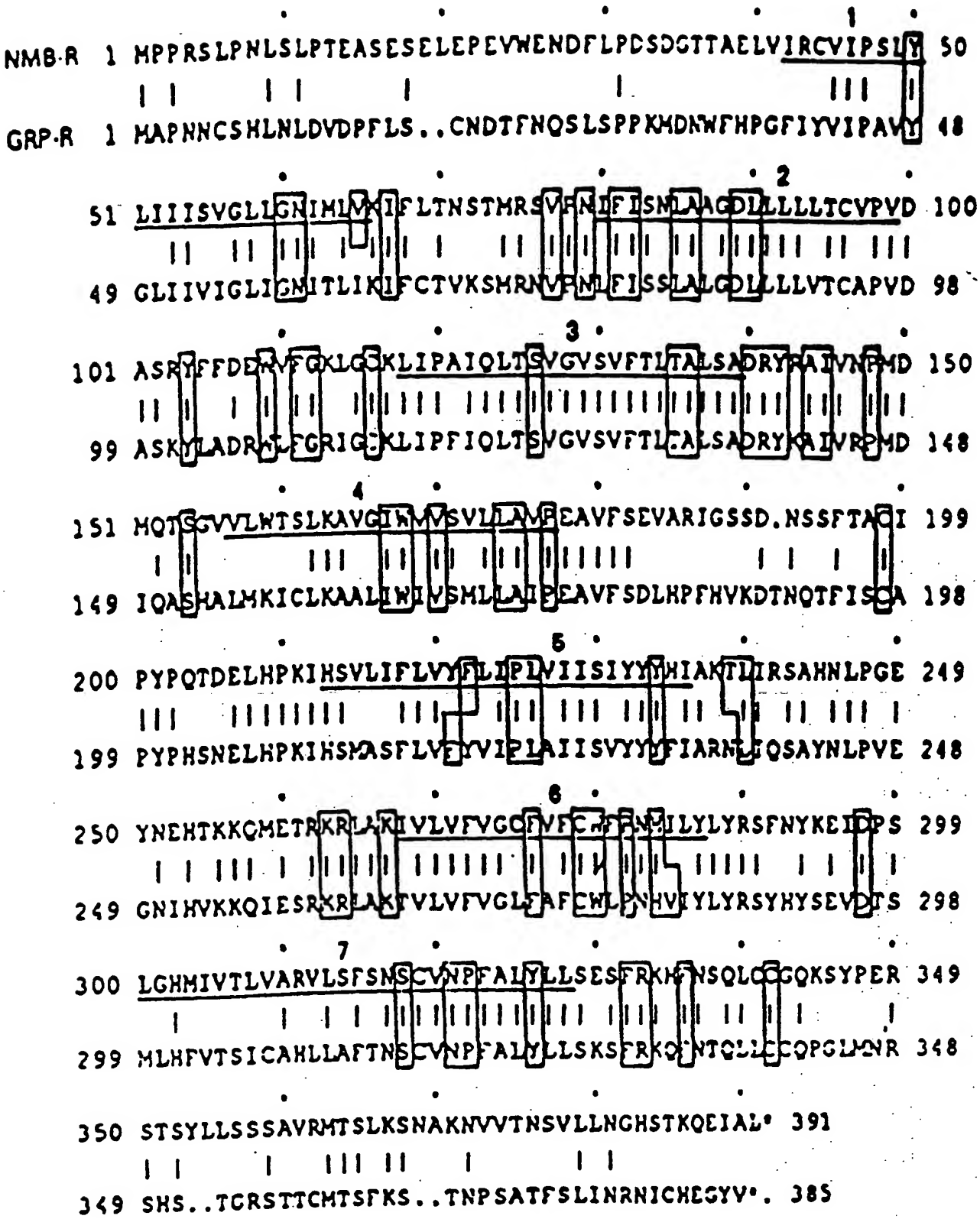
NMB-R 1 MPPRSLPNLSLPTEASESELEPEVWENDFLPDSDGTTAELVIRCVIPSLY 50
GRP-R 1 MAPNNCSHLNLDVDPFLS..CNDTFNQSLSPPKMDNWFHPGFIYVIPAVY 48
51 LIIISVGLLGNIMLVKIFLTNSTMRSVFNIFISMLAAGDLLLLLTCVPVD 100
49 GLIIVIGLIGNITLIKIFCTVKSMRNVFNLFISSLALGDLLLLVTCAPVD 98
101 ASRYFFDEWVFGKLGCKLIPAIQLTSVGVSVFTLTALSADRYRAIVNPMD 150
99 ASKYLADRWLFGRIGCKLIPFIQLTSVGVSVFTLCALSADRYKAIVRPMD 148
151 MQTGGVVLWTSLKAVGIWMVSVLLAVPEAVFSEVARIGSSD.NSSFTACI 199
149 IQASHALMKICLKAALIWIVSMLLAIPEAVFSDLHPFHVKDTNQTFISCA 198
200 PYPQTDELHPKIHSVLIFLVYFLIPLVIISIYYYHIAKTLIRSAHNLPGE 249
199 PYPHSNELHPKIHSMASFLVFYVIPLAIISVYYYFIARNLIQSAYNLPVE 248
250 YNEHTKKQMETRKRLAKIVLVFVGCFVFCWFPNHILYLYRSFNYKEIDPS 299
249 GNIHVKKQIESRKRLAKTVLVFVGLFAFCWLPNHVIYLYRSYHYSEVDTS 298
300 LGHMIVTLVARVLSFSNSCVNPFALYLLSESFRKHFNSQLCCGQKSYPER 349
299 MLHFVTSICAHLLAFTNSCVNPFALYLLSKSFRKQFNTQLLCCQPGLHNR 348
350 STSYLLSSSAVRMTSLKSNAKNVVTNSVLLNGHSTKQEIAL* 391
349 SHS..TGRSTTCMTSFKS..TNPSATFSLINRNICHEGYV*. 385

TABLE 7

A comparison between the amino acid sequence predicted for the NMB-R and other members of the G-protein coupled receptor superfamily shows that many amino acid residues conserved in this family are present at corresponding positions in the NMB-R sequence. Two cysteine residues that may form a disulfide linkage situated in the first and second extracellular loop are conserved in the predicted NMB-R sequence at positions 116 and 198. Another well conserved cysteine residue which is thought to be important in anchoring the beta-adrenergic receptor to the plasma membrane is also present in the predicted sequence of NMB-R, 14 amino acid residues downstream from the end of the seventh transmembrane domain. In addition, numerous other amino acid residues which are typically conserved in members of the G-protein coupled receptor superfamily are also found in the predicted amino acid sequence of the NMB-R (Table 7 boxed residues). These similarities indicate that, like the GRP-R, the NMB-R is a member of the G-protein coupled receptor superfamily.

EXAMPLE 17

Analysis of the Functional Properties of the NMB-R

To confirm the functional identity of the NMB-R cDNA, Xenopus oocytes were injected with RNA transcribed in vitro from cDNA clones containing the entire NMB-R protein coding domain. RNA was transcribed and capped in vitro from either the NMB-R or GRP-R cDNA clones using T7 RNA polymerase as recommended by the manufacturer (Promega). Defolliculated oocytes were microinjected with about 10 nanograms of mRNA per oocyte, and kept at 20° C in ND solution of Lupu-Meiri et al. (1989) Pflugers Arch. 413:498-504. After 24 to 48 hours, oocytes were placed in a perfusion chamber and voltage clamped at a holding potential of -60 mV. Ligands were added directly to the chamber and ligand-dependant Cl^- currents were measured. The GRP1-27 and NMB peptide were purchased from Peninsula (Burlingame, CA), and the [D-Phe^6]BN(6-13) ethyl ester antagonist was synthesized as described by Wang et al. (1990) J. Biol. Chem. 265:15695-15703.

Either NMB (10^{-6} M) or GRP (10^{-6} M) causes a depolarizing current which is typical for IP_3- and Ca^{+2}-mediated chloride channel opening. At lower agonist concentrations (10^{-9} M), only NMB could elicit a detectable response. These data establish that the cDNA clones isolated from the esophagus library encode a functional NMB-R that, in contrast to the GRP-R, responds to lower concentrations of NMB than GRP.

The effect of a specific antagonist for the GRP-R on the function of the NMB-R expressed in oocytes was tested. The des-Met bombesin analog ([D-Phe^6]BN(6-13) ethyl ester) functions as a specific antagonist for the pancreatic GRP-R but not the esophageal NMB-R. This antagonist completely blocks the electrophysiologic response of oocytes expressing the cloned Swiss 3T3 GRP-R when it is applied at a 10:1 molar ratio with micromolar concentrations of either GRP or NMB agonists. In contrast, addition of the antagonist along with either NMB or GRP agonist (10:1 molar ratio) did not diminish the response of the cloned NMB-R expressed in Xenopus oocytes.

To establish that the differences in physiological response of the receptor to NMB and GRP were due to relative binding affinities, the ligand binding properties of the cloned receptor expressed in Balb 3T3 fibroblasts were examined. Preliminary binding studies showed that Balb 3T3 cells would be an appropriate host for expressing the cloned NMB-R, since they have very low levels of endogenous displaceable bombesin binding.

An Eco RI fragment from the longest NMB-R cDNA clone encoding the entire open-reading frame was subcloned into a modified version of the pCD2 plasmid from Wada et al. (1989) Nature 342:684-689. Balb 3T3 cells were transfected with 40 micrograms of the NMB-R expression plasmid construct using the calcium phosphate precipitation method of Graham et al. (1973) Virology 52:456-467, with a few modifications, see Davis et al. (1986). Stably transfected cells were selected for resistance to the aminoglycoside G418 (800 μg/ml). After a three week selection period, 10 clones were screened for high affinity

binding. One cell line showing high levels of specific binding was selected for more detailed analysis.

Binding and displacement studies on the transfected Balb 3T3 cells were performed as described previously by Kris et al. (1987) J. Biol. Chem. 262:11215-11220, in 24 well tissue culture dishes using 25 pM ^{125}I-labeled bombesin purified after labeling by reverse phase high pressure liquid chromatography (von Schrenck et al. (1990) Amer. J. Physiol. 259:G468-G473). Each point on the displacement curve was determined four times, and the average value plotted. The bombesin displacement studies performed to determine the K_D values for NMB, GRP, and the ethyl ester antagonist on pancreatic and esophagus tissue sections were performed as described by von Schrenck et al. (1990).

The relative ligand affinity of the transfected NMB-R was assessed by quantitative displacement of ^{125}I-labeled bombesin (BN) binding by unlabeled NMB or GRP. NMB was more potent than GRP in displacing labeled BN (K_D for NMB = 2 nM; K_D for GRP = 43 nM). Ligand displacement properties determined for the transfected cells are compared in Table 8 to those obtained from esophageal tissue sections, known to express an NMB-R as well as the pancreatic acinar cell line AR42J, and pancreatic tissue sections known to express a GRP-R with properties similar to the Swiss 3T3 GRP-R. NMB was more potent than GRP in displacing ^{125}I-BN bound to transfected Balb 3T3 cells expressing the NMB-R, as was observed in esophagus tissue sections. In contrast, GRP is more potent than NMB in displacing ^{125}I-bombesin binding to pancreatic acinar cells, AR42J, or Swiss 3T3 cells. These results show that the cDNA under study encodes a functional NMB-preferring bombesin receptor, with binding properties resembling the esophageal NMB-preferring bombesin receptor reported previously. As expected, the specific GRP-R antagonist [D-Phe^6]BN(6-13) ethyl ester binds GRP-preferring receptors (pancreas, AR42J, Swiss 3T3) at high affinity (K_D = 1.6 to 5.3 nM), but has much lower affinity for NMB-preferring receptors on either esophagus or

Balb 3T3 cell expressing the cloned NMB-R (K_D > 1000 nM) (Table 8).

TABLE 8: Displacement of I-BN binding by GRP, NMB and [D-Phe^6]BN(6-13 ethyl ester antagonist in different BN receptor subtypes*

Cell Type	Ki (nM) NMB	GRP	antagonist
Balb 3T3/NMB-R	2	43	>1000
esophagus	0.3	30	>1000
pancreas	351	15	5.3
AR42J	287	2	2.1
Swiss 3T3	62	2	1.6

--

* Displacement of ^{125}I-BN binding GRP1-27, NMB, and a GRP-R antagonist [D-Phe^6]BN(6-13) ethyl ester was analyzed in tissues and cultured cells expressing different bombesin receptor subtypes. Whole cell binding studies on cell lines (Balb 3T3/NMB-R transfectants, Swiss 3T3) were performed essentially as described by Kris et al. (1987) J. Biol. Chem. 262, 11215-11220. Binding displacement analysis of tissue sections and AR42J cells was performed in a very similar manner, with a few modifications, to the method of von Schrenck et al. (1990) Amer. J. Physiol. 259:G468-G473. Binding properties of the NMB-R expressed on transfected Balb 3T3 fibroblast most closely resemble the esophagus NMB preferring receptor, and are clearly different from GRP preferring BN receptor subtypes found on pancreatic acinar cells, Swiss 3T3 cells, and AR42J cells.

EXAMPLE 18

Tissue Distribution of NMB-R mRNA

Bombesin receptors have been described in both neural and non-neural tissues, as well as various cell lines. To determine which cells express the NMB preferring bombesin receptor subtype encoded in the cDNA clone, mRNA was examined in various tissues and cell lines using Northern blot hybridization analysis. Poly $(A)^+$ RNA isolated from the rat brain, olfactory region, esophagus, and C6 glioma cell line each contain two hybridizing mRNA species present after a high stringency wash, with estimated sizes of approximately 3.2 kb and 2.7 kb. Both bands were observed together in all expressing tissues and were still present after high-stringency washing, suggesting that they are transcripts from the same gene. In contrast, no NMB-R mRNA was detected in poly $(A)^+$ mRNA samples isolated from pancreas, the AR42J rat pancreatic acinar cell line, and Swiss 3T3 cells, each shown previously to express GRP-R mRNA. No hybridizing mRNA species were detected by either the GRP-R or the NMB-R probe in mRNA samples from lung, thymus, and Balb 3T3 cells. These results show that the cloned NMB-R mRNA reported in this study is expressed in the brain as well as in the esophagus. NMB-R mRNA within the brain was localized to the olfactory bulb, a brain region reported to express relatively high levels of binding sites for NMB-preferring bombesin receptor.

EXAMPLE 19

NMB-R and GRP-R mRNA in Different Brain Regions

RNA blot hybridization studies on rat brain mRNA using both the NMB-R probe and the Swiss 3T3 GRP-R probe indicated that both bombesin receptor subtypes are expressed in the brain. NMB-R and GRP-R mRNA expression in the rat CNS was examined in more detail using *in situ* hybridization histochemistry to determine the correlation between regions expressing the specific cloned NMB-R and GRP-R genes, and regions shown in previous ligand binding autoradiographic studies to express brain bombesin binding sites. The method of

Wada et al. (1990) J. Neurosci. 10:2917-2930 was used for situ hybridization. Briefly, adult male rats were fixed by perfusion with 4% paraformaldehyde, 0.05% glutaraldehyde. After perfusion, the brain was removed and placed in post-fix solution (4% paraformaldehyde plus 10% sucrose) overnight at 4° C. Sections (25 micron thick) were mounted on polylysine-coated slides and then treated with proteinase K (10 g/ml, 37° C, 30 min), acetic anhydride, and dehydrated by successive immersion in 50%, 70%, 95%, and 100% ethanol. ^{35}S-labeled sense or antisense cRNA probes (specific activity about 2 X 10^9 cpm per microgram) were synthesized from a pGEM-4 plasmid vector (Promega) containing a 2.0 kb cDNA fragment encoding either the rat NMB-R or rat GRP-R subcloned in the polylinker region between the SP6 and T7RNA polymerase promoters. Hybridizations were performed in 50% formamide, 0.3 M NaCl, 10% dextran sulfate, 10 mM DTT at 55° C overnight, with a probe concentration of 5 X 10^6 cpm per ml of hybridization buffer. Sections were then washed in a solution containing 4 X SSC (1 x SSC = 150 mM NaCl, 15 mM NaCitrate pH 7.0) and 1 mM DTT at room temperature, incubated with RNAse A (20 μg/ml at 37° C for 30 min), and washed at room temperature with solutions containing progressively lower concentrations of SSC and 1 mM DTT, beginning with 2 X SSC and ending with 0.5 X SSC. A final high stringency wash was performed in a solution containing 0.1 X SSC and 1 mM DTT at 55° C for 30 min. Slides were dehydrated in 50%, 70%, 95%, and 100% ethanol and exposed to βmax film (Amersham) at room temperature for 3-7 days.

Probes were hybridized to coronal rat brain sections from the olfactory regions as well as thalamic and hypothalamic regions where labeled bombesin and NMB binding were prominent in previous studies. Overall, NMB-R expression was most striking in the olfactory and central thalamic regions, while GRP-R expression was most prominent in the hypothalamus. More detailed analysis of the sections showed the NMB-R mRNA expression was highest in the anterior olfactory nucleus, tenia tecta, and piriform cortex. In addition, many other regions, including the accessory olfactory bulb, frontal cortex,

thalamic nuclei (paraventricular, antero dorsal, centromedial, centrolateral, and rhomboid), dentate gyrus, amygdalopiriform nucleus, and dorsal raphe also expressed NMB-R. GRP-R mRNA expression was highest in the suprachiasmatic nucleus, paraventricular nucleus, nucleus of the lateral olfactory tract, magnocellular preoptic nucleus, and lateral mammillary nucleus. Moderate expression was seen in the bed nucleus of the accessory olfactory tract, lateral hypothalamic area, supraoptic nucleus, dentate gyrus, field CA3 of Ammon's horn, isocortex, medial amygdaloid nucleus, and nucleus ambiguous. These results show that NMB-R and GRP-R mRNAs are selectively expressed in different rat brain regions. Similar selective expression should be found in other species.

EXAMPLE 20

Isolation and Characterization of Human Genomic and cDNA GRP-receptor Clones

To determine the germline sequence of the human GRP-R, a placental genomic library was screened using the coding region of the Swiss 3T3 GRP-R cDNA as a probe.

Approximately 1 x 10^6 recombinants from a human-placenta genomic library (Stratagene, La Jolla, CA) were screened with a ^{32}P-labeled Swiss 3T3 GRP-R probe containing the coding region. Filter hybridization was at 37° C using previously described methods of (Davis et al. (1986). Filters were washed twice at room temperature for 15 minutes in 300 mM NaCl, 30 mM NaCitrate, 0.1% sodium dodecyl sulfate (SDS), and at 50° C twice for 15 minutes in 15 mM NaCl, 1.5 mM NaCitrate, 0.1% SDS. Positive clones were plaque purified and smaller hybridizing fragments subcloned into pGEM4 (Promega, Madison, WI) and sequenced.

After identifying the 3'-untranslated region of the genomic human GRP-R clone, a primer was synthesized from this region and used to prime first strand cDNA synthesis from NCI-H345 oligo-dT cellulose selected mRNA by methods previously described in Davis et al. (1986). The NCI-H345 cell line is a GRP-responsive SCLC cell line, see Cuttitta et al. (1985)

Nature 316:823-825. From this library four positive clones were plaque purified and sequenced. The 1152 nucleotides determining the protein coding region sequence of these clones from SCLC were found to be identical to those of the exons found in the genomic human GRP-R sequence. This result indicates that the GRP-R protein coding sequence is unaltered in this SCLC cell line.

The sequence of the human GRP-R coding region is illustrated in Table 2. The human GRP-R is contained in three exons, and the predicted amino acid sequence encodes a 384-amino acid protein which is identical in length to that which has been determined for the Swiss 3T3 mouse GRP-R. Comparison of the amino acid sequence derived from the human clone to that of the mouse Swiss 3T3 sequence demonstrated a 90% amino acid identity (vertical lines in Table 9). There is far less conservation at the amino terminus of the GRP-R protein between mouse and human (Table 9). Hydropathy analysis of the predicted human GRP-R protein, see Figure 13, reveals seven regions of hydrophobic amino acids, consistent with a seven transmembrane structure typical of G-protein coupled receptors (see Dohlman et al. (1987) Biochemistry 26:2657-2663). There are also four conserved consensus sites of potential protein kinase C phosphorylation (see Kishimoto et al. (1985) J. Biol. Chem. 260:12492-12499; Woodgett et al. (1986) Eur. J. Biochem. 161:177-184) (asterisks over potential phosphorylation sites in Table 9).

Table 9: Comparison of the derived amino acid sequences for the mouse Swiss 3T3 (upper sequence) and the human GRP-R (lower sequence). Overall amino acid identity was 90%, indicated by vertical lines. Numbered bold lines above amino acids show the location of seven predicted hydrophobic transmembrane domains. Asterisks indicate conserved sites for protein kinase C phosphorylation.

```
                                                        1
  1 MAPNNCSHLNLDVDPFLSCNDTFNQSLSPPKMDNWFHPGFIYVIPAVYGL 50
    || | |  ||| || |  ||     |   | | | |||  ||||||||
  1 MALNDCFLLNLEVDHFMHCNIS.SHSADLPVNDDWSHPGILYVIPAVYGV 49

                                  *  *          2
 51 IIVIGLIGNITLIKIFCTVKSMRNVPNLFISSLALGDLLLLVTCAPVDAS 100
    || ||||||||||||||||||||||||||||||||||||| ||||||||
 50 IILIGLIGNITLIKIFCTVKSMRNVPNLFISSLALGDLLLLITCAPVDAS 99

                          3
101 KYLADRWLFGRIGCKLIPFIQLTSVGVSVFTLTALSADRYKAIVRPMDIQ 150
    ||||||||||||||||||||||||||||||||||||||||||||||||||
100 RYLADRWLFGRIGCKLIPFIQLTSVGVSVFTLTALSADRYKAIVRPMDIQ 149

                    4
151 ASHALMKICLKAALIWIVSMLLAIPEAVFSDLHPFHVKDTNQTFISCAPY 200
    ||||||||||||| ||| ||||||||||||||||   ||||||||||
150 ASHALMKICLKAAFIWIISMLLAIPEAVFSDLHPFHEESTNQTFISCAPY 199

                          5
201 PHSNELHPKIHSMASFLVFYVIPLAIISVYYYFIARNLIQSAYNLPVEGN 250
    |||||||||||||||||||||| |||||||||| ||||||||||||||
200 PHSNELHPKIHSMASFLVFYVIPLSIISVYYYFIAKNLIQSAYNLPVEGN 249

                  *       6
251 IHVKKQIESRKRLAKTVLVFVGLFAFCWLPNHVIYLYRSYHYSEVDTSML 300
    ||||||||||||||||||||||||||||||||||||||||||||||||||
250 IHVKKQIESRKRLAKTVLVFVGLFAFCWLPNHVIYLYRSYHYSEVDTSML 299

                    7
301 HFVTSICAHLLAFTNSCVNPFALYLLSKSFRKQFNTQLLCCQPGLMNRSH 350
    |||||||| |||||||||||||||||||||||||||||||||  |||
300 HFVTSICARLLAFTNSCVNPFALYLLSKSFRKQFNTQLLCCQPGLIIRSH 349

                *
351 STGRSTTCMTSFKSTNPS.ATFSLINRNICHEGYV* 385
    |||||||||||| |||||| ||||||| ||||| |||
350 STGRSTTCMTSLKSTNPSVATFSLINGNICHERYV* 385
```

EXAMPLE 21

Functional Evaluation of GRP-receptor cDNA

To evaluate the function and pharmacology of the cloned NCI H345 human GRP-R cDNA, Xenopus oocytes were injected with an in vitro transcript encompassing the coding region of the NCI-H345 GRP-R cDNA.

Functional Expression of Human GRP-R in Xenopus Oocytes

RNA was transcribed and capped in vitro from the GRP-R cDNA clone using T7 RNA polymerase as recommended by the manufacturer (Promega). Defolliculated oocytes were microinjected with approximately 10 nanograms of mRNA per oocyte, and kept at 20° in ND solution of Lupu-Meiri et al. (1989) Pflugers Arch. 413:498-504. After 24 to 48 hours, oocytes were placed in a perfusion chamber and voltage clamped at a holding potential of -60 mV. Ligands were added directly to the chamber, and ligand dependent Cl^- currents were measured.

GRP applied at nanomolar concentrations was shown to elicit a depolarizing response in oocytes injected with the transcript. This response was shown to be blocked by an antagonist specific for the GRP-R, ([D-Phe^6]BN(6-13) ethyl ester) at a 10:1 molar ratio of antagonist:agonist. Taken together, these data indicate that the cDNA isolated from NCI-H345 does encode a functional GRP-R that is functionally and pharmacologically indistinguishable to that isolated from Swiss 3T3 cells.

EXAMPLE 22

Analysis of the Expression of GRP-receptor mRNA by Northern blot and RNase protection analysis

Expression of GRP-R mRNA was examined in the SCLC cell line, NCI-H345, by Northern blot analysis. The predominant hybridizing mRNA species in this cell line had an estimated size of 3.1 kb. The human GRP-R probe also hybridized to two sizes of mRNA from Swiss 3T3 cells (approximately 7.2 kb and 3.1 kb). The level of GRP-R mRNA

observed in NCI-H345 was low, near the threshold of detection. Since RNA blot analysis might fail to detect low but significant levels of GRP-R mRNA, a more sensitive RNase protection assay was used to detect GRP-R mRNA in a panel of SCLC and non-SCLC lung cancer cell lines.

Lung cancer cell lines were obtained from Dr. J. Minna and Dr. A. Gazda. These cells were established and typed histologically as described, e.g., in Carney et al. (1985) Cancer Res. 45:2913-2919; Brower et al. (1986) Cancer Res 46:798-806; Carmichael et al. (1988) Br. J. Cancer 58:437-440; Harbour et al. (1988) Science 241:353-357; and Takahashi et al. (1989) Science 246:491-493. Total RNA was isolated from cells using guanidine thiocyanate homogenization and CsCl gradient purification as described by Davis et al. (1986). The probe for this assay was transcribed with T7 polymerase from a Bgl II-Hind III 600 bp genomic fragment cloned into pGEM4 according to the manufacturers directions (Promega). DNA template was removed by digestion with 5 units RQ1 DNase (Promega). Unincorporated nucleotides in the resulting reaction were removed by multiple ethanol precipitations and the resulting pellet was resuspended in 10 mM TRIS-HCl, pH 7.4; 1 mM DTT. The probe was diluted to a concentration of 2.5×10^5 cpm/μl. RNA samples to be hybridized (30 μl) were dried and resuspended in 50 μl hybridization mix (20 mM TRIS-HCl, pH 7.4; 500 mM NaCl; 2 mM EDTA; 78% formamide; 1 μl, 2.5×10^5 cpm GRP-R probe). The samples were heated to 80° C for 2 minutes and hybridized 16-18 hours at 43° C.

Unprotected RNA was digested in a reaction consisting of 88 units RNase A (United States Biochemical); 20 mM TRIS-HCl, pH 7.4; 300 mM NaCl; and 1 mM EDTA in a final volume of 350 μl at 37° C for 30 minutes. The reaction was then made 0.5% in SDS and 0.05 μg of proteinase K (BRL) was added and incubated at 37° C for 15 minutes. The reaction was then extracted with phenol/chloroform and ethanol precipitated. The pellet was collected by centrifugation and resuspended in 5 μl of the following solution: 80% formamide; 50 mM TRIS; 50 mM borate; 11 mM EDTA; 0.1% Bromophenol Blue; 0.1% Xylene Cyanol.

Samples were denatured for 2 minutes at 95° C prior to electrophoresis on a 6% denaturing polyacrylamide gel. The gel was dried and exposed to X-ray film in the presence of an intensifying screen.

The GRP-R probe used above was derived from a human genomic GRP-R clone which included 299 bp of exon 2 (nucleotides 465-764, Table 2) and extended 301 bp into the second intron. Accordingly, the probe would be protected from ribonuclease digestion by a 299 base region of the GRP-R mRNA.

GRP-R mRNA was detected in cell lines from all histological types of lung carcinoma examined, but not all members of any one histological group were found to express GRP-R mRNA. Data from various lung carcinoma cell lines is summarized in Table 10. A representative autoradiograph of the assay results is shown in Figure 19 and described in more detail in Example 26. Additionally, the level of GRP-R message varied among expressing cell lines. The highest level of expression was found in the SCLC cell line NCI-H345.

Table 10: GRP- and NMB- receptor mRNA levels in lung cancer cell lines as determined by RNase protection assay. Signal strength on resulting autoradiogram was assessed and assigned an arbitrary value relative to other cell lines. See also description in Example 26.

Cell line and morphological type	GRP-receptor	NMB-receptor
Small Cell Lung Carcinoma		
NCI-H60	+	
NCI-H69	tr	-
NCI-H82	-	-
NCI-H146	tr	
NCI-H187	-	
NCI-H209	-	++
NCI-H345	++	++
NCI-N417	-	-
NCI-N510	tr	+
NCI-N592	+	-
NCI-H889	+	
NCI-H1092	+	
Carcinoid		
NCI-H720	+	-
NCI-H727	+	-
Non-Small Cell Lung Carcinoma		
NCI-H23	-	-
NCI-H125	tr	-
NCI-H157	-	-
NCI-H226	-	-
NCI-H322	+	-
NCI-H358	-	-
NCI-H441	-	-
NCI-N460	-	-
NCI-H520	+	-
NCI-H661		-
NCI-H810		-
NCI-H1299		+
NCI-H1373		+

EXAMPLE 23

Pharmacological Evidence for Distinct Receptors for Bombesin-like Peptides

Bombesin-like peptides induce an increase in intracellular calcium in the NCI-H345 cell line. Bombesin-stimulated Ca^{2+} mobilization studies were performed in the human lung carcinoma cell line NCI-H345 using Quin 2-fluorescence in order to determine if one or more bombesin receptor subtypes could be active in these cells.

NCI-H345 SCLC cells were cultured in SIT medium (RPMI 1640, (GIBCO) with 10 mM HEPES (pH 7.4) and 30 nM sodium selenite, 5 μg/ml insulin, and 10 μg/ml transferrin). Cells were washed three times in 0.015 M $NaPO_4$, 0.15 M NaCl, 0.01 M HEPES, pH 7.4, and once in SIT medium. The washed cells were suspended in SIT medium at 1 X 10^7 cells/ml. These cells were incubated with 5 μM of quin-2-acetoxymethyl ester (quin-2; Molecular Probes, Eugene, OR) at 37° C for 90 minutes. After incubation the cells were washed once and resuspended in SIT medium without quin-2 at 1 X 10^7 cells/ml. Approximately, 5 X 10^6 cells were pelleted and resuspended in 2 ml of HEPES buffered saline (140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, and 20 mM Hepes, pH 7.4) in an Elkay Lab Systems acrylic "ultra-UV", four-sided, 10 mm, 4.5 ml cuvette. Using a Perkin Elmer L5B Luminescence Spectrometer, with an excitation wavelength of 339 nm and an emission wavelength of 492 nm (slits = 5 nm) the change in fluorescence after addition of ligand was measured. The cells in the cuvette were kept at a constant temperature (37° C) and were continuously suspended with a magnetic stirrer while the fluorescent measurements were taken. Ligand was added to the cells when a stable fluorescence reading was obtained, usually within 5 minutes. The inhibitor [D-Phe^6]BN(6-13) ethyl ester was added to the cells five minutes prior to the addition of ligand. To determine total $[Ca^{2+}]_i$ the cells were lysed by addition of 10 μl 10% Triton-X to obtain F_{max}. Then, 100 μl 0.4 M EGTA was added to the cuvettes to determine the fluorescence background (F_{min}). The $[Ca^{2+}]_i$ was calculated from the fluorescence measurements using the formula:

$$[Ca^{2+}]_i = \frac{(F_{observed} - F_{minimum}) \times 115\ nM}{(F_{maximum} - F_{observed})}$$

Both bombesin and NMB elicited an immediate calcium response in these cells (Figure 14). In several experiments, the increase in intracellular calcium mediated by NMB was consistently more sustained than that elicited by Tyr^4-bombesin. The increase in intracellular calcium was detected at <1 nM concentrations of NMB agonist, and maximal at about 100 nM for both NMB and Tyr^4-bombesin. Either peptide alone could elicit a detectable response at between 1 and 10 nM levels (Figure 15). These observations indicate that at least part of the calcium mobilization response is mediated by a bombesin receptor subtype that binds NMB at high affinity, pharmacologically similar to the esophageal NMB-R.

Figure 16 shows that approximately 50% of the increase in intracellular calcium elicited by Tyr^4-bombesin is blocked by the GRP-receptor specific antagonist, [D-Phe^6]BN(6-13) ethyl ester at 30 nM concentrations, whereas further inhibition of the BN-mediated calcium response is complete only after the addition of 1000 to 10,000 nM antagonist. The NMB-elicited calcium response was insensitive to the antagonist (minimal effects on calcium response at >1000 nM concentrations, as shown in Figure 16). These data further demonstrate that the calcium response to bombesin-like peptides in NCI-H345 is mediated by at least two distinct receptors, and that both the human GRP-preferring and NMB-preferring bombesin receptors are expressed and functional in human lung carcinoma cells.

Bombesin-like peptides are expressed in human SCLC and are thought to function as autocrine growth factors. These results show that the SCLC cell line NCI-H345 expresses two pharmacologically distinct bombesin-peptide receptors one of which is GRP-preferring and blocked by the antagonist, [D-Phe^6]BN(6-13) ethyl ester and the other which is NMB preferring and was not blocked by the antagonist. A subset of lung

carcinoma cell lines examined express either receptor, or both receptors, at levels detectable by a sensitive RNase protection assay, but often below the level of detection by Northern blot analysis of total RNA, see discussion below, Example 26. The low levels of GRP-R and NMB-R mRNA are consistent with bombesin ligand binding studies in lung carcinoma cell lines which showed less than 5000 receptors per cell.

EXAMPLE 24

Isolation of Human NMB Receptor

The bombesin-stimulated calcium mobilization properties indicated that more than one bombesin receptor subtype exists in NCI-H345. Thus, distinct human GRP-R and NMB-R receptor cDNA clones should be isolatable using murine Swiss 3T3 GRP-R cDNA, see Battey et al. (1991) Proc. Natl. Acad. Sci. USA 88:395-399, or rat NMB-R cDNA, see Wada et al. (1991) Neuron 6:421-430, as probes. The isolation of human GRP-R is described in Example 20. Briefly, human genomic NMB-R clones were isolated from both placental and peripheral blood genomic libraries to compare the sequence of receptor cDNA clones derived from the NCI-H345 tumor cells with their normal genomic counterparts.

Isolation of human genomic and cDNA clones

Approximately 1 X 10^6 recombinants from a human-placenta genomic library (Stratagene, La Jolla, CA) and a human-peripheral blood genomic library (Promega, Madison, WI) were screened with a ^{32}P-labeled rat neuromedin-B probe containing the coding region. The general procedure described above, see Example 20, for isolating the human GRP receptor was followed.

To obtain a human neuromedin B receptor cDNA, oligonucleotides (5' sense primer: 5'GTGGGCGTTCAGTCCTCAGG 3'; 3' antisense primer: 5'GTTCTCTCCAGGTAGTGAGTT 3') complementary to sequences from the 5'- and 3'-untranslated domains that immediately flank the coding region were synthesized for use as polymerase chain reaction (PCR) primers. These primers were

then used in PCR with 20 ng hexamer primed cDNA template reverse transcribed from poly-A+ NCI-H345 mRNA. Buffers and nucleotides were provided in the GeneAmp PCR kit (Perkin-Elmer). The cycling conditions were: 94° C, 1 min; 60° C, 1 min; 72° C, 2.5 min. for 40 cycles. The ends of the resulting products were polished with T_4 DNA polymerase, and the 5'-ends phosphorylated with T_4 polynucleotide kinase to allow subcloning into the 5' dephosphorylated Sma I site of pGEM-4. Positive colonies were identified by hybridization to the rat neuromedin-B receptor probe. Two clones were sequenced.

The entire amino acid coding sequences of the human NMB receptor genomic clones were sequenced on both strands using gene-specific synthetic oligonucleotide primers. See Table 4. Nucleotide sequence analysis was performed using the Sequence Analysis Software Package (Pepplot program for the hydropathy analysis) of the University of Wisconsin Genetics Group and a VAX computer. See Devereux et al. (1984) Nucleic Acids Res. 12:387-395. A hydropathy analysis is shown in Figure 17.

The human GRP-R coding region is contained in three exons, and the predicted amino acid sequence encodes a 384-amino acid protein as described above. The human NMB-R is also contained in three exons, and the predicted amino acid sequence encodes a 390-amino acid protein. Analysis of two NMB-R cDNA clones isolated from NCI-H345 revealed that the protein coding region sequence of these clones was identical to the sequence of the exons found in the human genomic NMB-R gene. A similar comparison of GRP-R sequences from normal and SCLC cell lines is reported above, and shows the same identity. Thus, neither the GRP-R or NMB-R protein coding sequence is structurally altered by somatic mutation in this SCLC cell line.

Molecular Genetic Analysis of human GRP-R and NMB-R

Both human GRP-R and NMB-R coding regions show high amino acid identity with their rodent counterparts (GRP-R 90% identity, NMB-R 89% identity). Hydropathy analysis of the predicted GRP-R and NMB-R proteins reveals seven regions of hydrophobic amino acids (Tables 2 and 4; Table 11, boxes)

consistent with a seven transmembrane structure typical of G-protein coupled receptors. Comparison of the human GRP-R and NMB-R sequences indicates 55% identity at the amino acid level (vertical lines, Table 11). There are also two consensus sites of potential protein kinase C phosphorylation in both GRP-R and NMB-R (dotted outline boxes enclose potential phosphorylation sites in Table 11). Of interest, the two introns that divide the protein coding region are found in analogous locations in both the GRP-R and NMB-R genes (Table 1 and 4), suggesting that both receptor genes evolved by duplication of a common ancestor.

Several structural features of the human GRP-R and NMB-R are worthy of note. Comparison of the predicted amino acid sequences of human GRP-R and human NMB-R (Table 11 shows that the third transmembrane domain is extremely well conserved between these two receptor subtypes; 95% identical in this region versus 55% identity for the entire amino acid sequence). In contrast, this domain is not particularly well conserved (<25% identity) when compared to other known G-protein coupled receptors. These results suggest that this region may be involved in ligand binding, or other functional properties that would be expected to be similar among closely related receptor subtypes but not common to all members of the G-protein coupled receptor family. The genomic sequences of NMB-R and GRP-R show that the first intron is located at the same position in both genes, immediately carboxy-terminal to the third transmembrane domain (Tables 2 and 4; Asp Arg Tyr). Several other intron-containing G-protein coupled receptor genes, e.g., substance P receptor, D_2 and D_3 dopamine receptors, and opsins, also contain an intron at this location, e.g. Asp Arg Tyr. This conserved structural feature suggests that these members of the G-protein coupled receptor superfamily evolved from a common ancestor.

Table 11: Comparison of the derived amino acid sequences from a human GRP-R (upper sequence) and a human NMB-R (lower sequence). Overall amino acid identity was 55% (indicated by vertical lines). Shaded boxes indicate the location of seven predicted hydrophobic transmembrane domains. Dotted-outline boxes enclose conserved potential sites of protein kinase C phosphorylation.

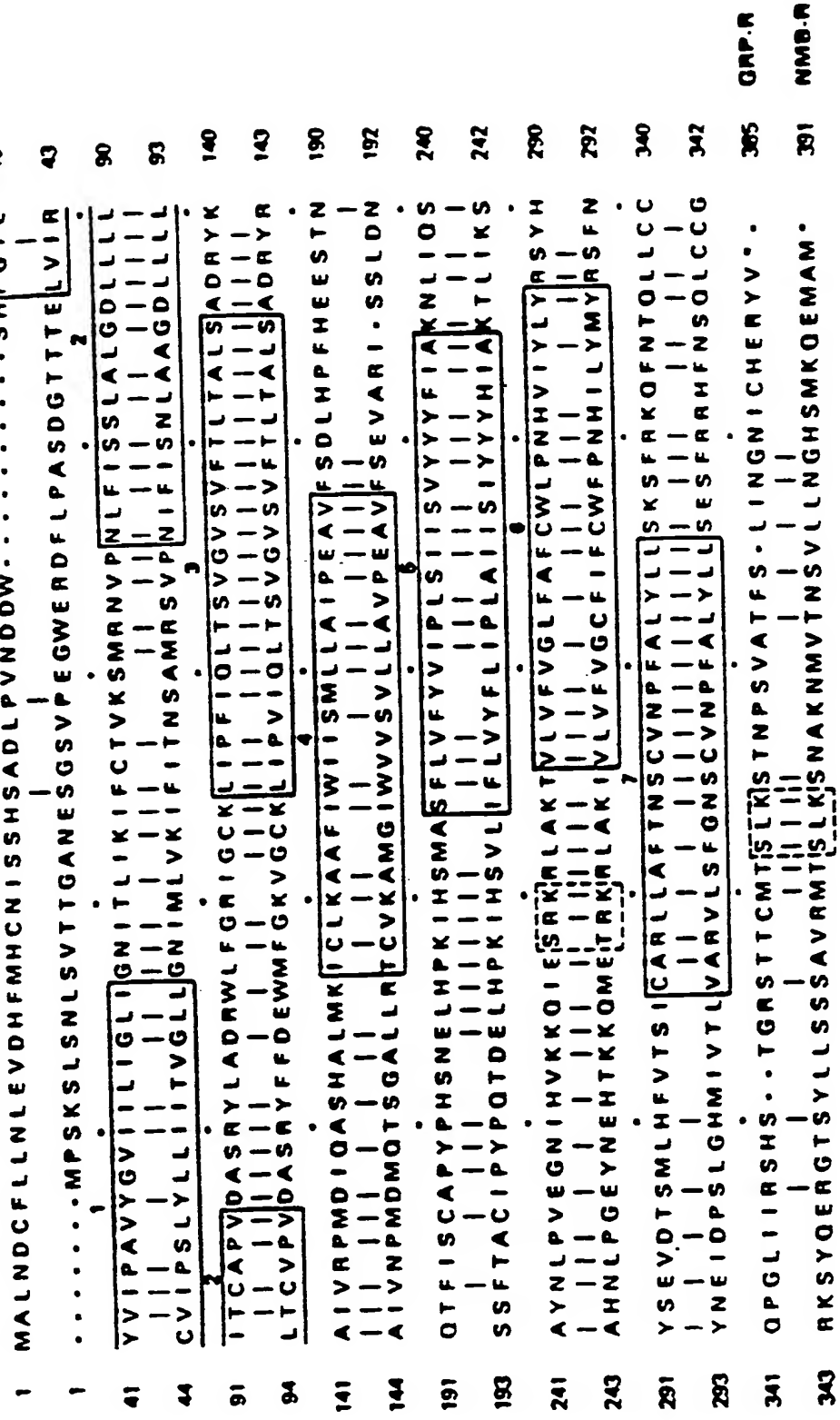

TABLE II

EXAMPLE 25
Functional Comparison of Cloned Human GRP Receptor and NMB Receptor

To evaluate the functional properties and pharmacology of the cloned NCI-H345 human GRP-R and NMB-R, Xenopus oocytes were injected with an in vitro transcript encompassing the coding region of either the NCI-H345 GRP-R or NMB-R cDNA.

RNA was transcribed and capped in vitro from the GRP-R and NMB-R cDNA clones using T7 or SP6 RNA polymerase as recommended by the manufacturer (Promega). Defolliculated oocytes were microinjected with approximately 10 nanograms of mRNA per oocyte, and kept at 20° C in ND solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM Na^+HEPES, 1.8 mM $CaCl_2$). After 24 to 48 hours, oocytes were placed in a perfusion chamber and voltage clamped at a holding potential of -60 mV. Ligands were added directly to the chamber, and ligand-dependant Cl^- currents were measured.

In oocytes injected with approximately 10 ng of the GRP-R transcript, GRP applied at 10^{-8} M concentration consistently elicited a depolarizing response which was greater in magnitude than the response to 10^{-8} M NMB (Figure 18A). This response was blocked by an antagonist specific for the GRP-R, ([D-Phe^6]BN(6-13) ethyl ester) at a 10:1 molar ratio of antagonist:agonist as shown in Figure 18A. In contrast, oocytes injected with NMB-R transcript showed a greater response to 10^{-8} M NMB than to an equivalent concentration of GRP (Figure 18B). The responses of oocytes injected with NMB-R were not blocked by that GRP-receptor specific antagonist, [D-Phe^6]BN(6-13) ethyl ester (Figure 18B). These results are consistent with previous studies of rodent bombesin receptor subtypes. The oocyte expression studies of cloned GRP-R and NMB-R isolated from NCI-H345 are consistent with the properties of the Ca^{2+} response elicited by bombesin peptide agonists in intact NCI-H345 cells, where both an antagonist-sensitive response to bombesin and an antagonist-insensitive NMB response were observed (Figure 14).

Repeated application of bombesin peptide agonists results in a rapid desensitization of the responses mediated through either the GRP-R or NMB-R expressed in Xenopus oocytes, or the calcium mobilization response to bombesin observed in NCI-H345. In a previous study of bombesin receptor function in SCLC, the phorbol compound PMA, which activates protein kinase C (PK-C), had no effect on the intracellular Ca^{++} concentration in the SCLC cell line NCI-H345, but attenuated the bombesin-stimulated increase in intracellular Ca^{++}. It has been demonstrated that the early cellular responses following stimulation of the Swiss 3T3 GRP receptor by ligand included activation of protein kinase C, as demonstrated by bombesin-stimulated phosphorylation of an 80 kDa protein substrate for PK-C. Taken together, these observations suggest that bombesin receptors are phosphorylated at PK-C recognition sites present in the receptor protein after receptor activation, and that phosphorylation of these sites may desensitize the receptor to subsequent activation. Notably, two consensus PK-C phosphorylation sites are conserved in both the human GRP-R and NMB-R sequences (Table 11, dotted outline boxes) in segments of the protein predicted to be intracellular (third cytoplasmic loop and carboxy terminal domain).

PK-C mediated phosphorylation of one or both of these sites may provide a mechanism to transiently desensitize the receptor. Studies using site-directed mutagenesis of the GRP-R cDNA and NMB-R cDNA to alter these sites are described in Example 28, below.

EXAMPLE 26

RNAse Protection Analysis Comparing the Expression of NMB-R mRNA in Lung Carcinoma Cells

Since NCI-H345 lung carcinoma cells express both functional GRP-R and NMB-R, the patterns of expression for both receptors in a panel of other lung carcinoma cell lines were also examined. GRP-R and NMB-R mRNAs are relatively rare transcripts in NCI-H345 mRNA, detectable by RNA blot analysis only after long autoradiographic exposures. To detect low but significant levels of GRP-R mRNA and NMB-R mRNA, a more sensitive RNase protection assay as described in Example 22 was used to analyze lung carcinoma mRNA samples for expression of these peptide receptors.

Northern Blot Analysis

Total RNA (10 μg) was resolved by electrophoresis on agarose/formaldehyde gels, and blotted to nitrocellulose membranes using methodology of Davis et al. (1986). After baking at 80^{o} C, membranes were hybridized to a ^{32}P-labeled human beta-actin fragment that contained the entire coding region. Blots were washed at high stringency (65^{o} C in 15 mM NaCl, 1.5 mM sodium citrate, 0.1% SDS, for two cycles of 15 minutes each).

RNAse Protection Assay

The assay was performed according to the procedure described above for the GRP-R transcripts.

Lung cancer cell lines were obtained from Dr. J. Minna and Dr. A. Gazdar. Total RNA was isolated from cells using guanidine thiocyanate homogenization and CsCl gradient purification according to Davis et al. (1986) and as described above. The NMB-R probe was a 400 bp Hind III genomic fragment. The fragment was cloned into pGEM-4 and transcribed according to the manufacturers directions (Promega). DNA template was removed by digestion with 5 units RQ1 DNase (Promega). Unincorporated nucleotides in the resulting reaction were removed by multiple ethanol precipitations and the resulting pellet was resuspended in 10 mM TRIS-HCl, pH 7.4; 1 mM DTT. The probe was diluted to a concentration of 2.5 x 10^{5} cpm/μl. RNA samples to be hybridized (30 μg) were dried and resuspended

in 50 μl hybridization mix (20 mM TRIS-HCl, pH 7.4; 500 mM NaCl; 2 mM EDTA; 78% formamide; 1 μl, 2.5 x 10^5 cpm NMB-R probe). The samples were heated to 80° C for 2 minutes and hybridized 16-18 hours at 43° C.

The NMB-R probe used in the RNase protection assay was an approximately 400 bp Hind III fragment of the human genomic NMB-R clone that contained a portion of the second intron and extended 219 bp (nucleotides 771-990, Table 4) into the third exon. Therefore the probe would be protected by a 219 base region of the NMB-R mRNA.

A representative autoradiograph indicating the results of this assay is shown in Figure 19 (Fig 19A, GRP-R; Fig 19B, NMB-R) and the data from all lung carcinoma cell lines examined are summarized in Table 10. GRP-R mRNA was detected in 10 of 22 cell lines from all histological types of lung carcinoma examined. See Table 10. Not all SCLC cell lines express GRP-R (4 of 7). Additionally, the level of GRP-R mRNA varied among expressing cell lines. The highest level of expression was found in the SCLC cell line NCI-H345. NMB-R expression was expressed in 5 of 22 lung carcinoma cell lines, with highest levels found in NCI-H209. Expression of one receptor subtype did not exclude expression of the other subtype; both SCLC line NCI-H345 and NCI-H510 express both GRP-R and NMB-R mRNA.

Molecular genetic studies of the structure of growth regulatory genes in human lung cancer cells frequently showed evidence of somatic mutation or gene deletion which alters the regulation or function of the encoded protein. The nucleotide sequence of several GRP-R and NMB-R cDNA clones isolated from the SCLC cell line NCI-H345 are identical to the sequence of the respective genomic clones for these receptors throughout the protein coding region. Thus, the GRP-dependent growth stimulation observed in lung cancer cells does not require a structural change in the GRP-R protein or in the NMB-R protein, i.e., the natural receptor is present and expressed.

Instead, it seems more likely that malignant cells may be stimulated to grow by the normal intracellular signals

evoked by ligand-dependant activation of bombesin-like peptide receptors. It has been reported that many different putative G-protein coupled neuropeptide receptors, e.g., vasopressin, bradykinin, cholecystokinin, galanin, and neurotensin, can transiently increase intracellular calcium in SCLC. A previous study shows that individual SCLC cell lines have great heterogeneity in response to a particular neuropeptide, but great similarity in possessing the capacity to increase intracellular calcium in response to at least one neuropeptide. Receptors for these neuropeptides are all G-protein coupled, and potentially activate a similar signal transduction pathway which may be important to the growth or cellular economy of SCLC.

The antagonist [D-Phe^6]BN(6-13) ethyl ester at 500 nM concentrations only partially inhibits the calcium response elicited by 50 nM [Tyr^4]BN in NCI-H345 SCLC cells, consistent with the conclusion from molecular genetic studies that the bombesin response is mediated by both the antagonist sensitive GRP-R and the relatively insensitive NMB-R. It is noted that very high concentrations of antagonist (10 nM) can completely block the NCI-H345 calcium response to 50 nM [Tyr^4]BN, while similar high levels of antagonist do not block responses elicited from the cloned NMB-R expressed alone in Xenopus oocytes under similar circumstances. The explanations for this difference in sensitivity is not clear at present. The co-expression of GRP-R and NMB-R in some way probably increases the antagonist sensitivity of the NMB-R mediated calcium response to [Tyr^4]BN in the NCI-H345 cells. Additional studies of the nature of responses elicited by bombesin peptides in cells expressing both GRP-R and NMB-R will determine whether or not the two receptors appear to generate responses independently, or interact in some more complex fashion.

Although GRP ligand expression is confined to SCLC cell lines, GRP-R and NMB-R mRNA expression is not restricted to SCLC lung carcinoma cell lines. Since these non-SCLC cell lines do not express preproGRP mRNA, autocrine growth stimulation of the GRP-R seems unlikely in these non-SCLC cell

lines. Elevated levels of bombesin-like peptides have been noted in the bronchial secretions of heavy smokers. Bombesin-like peptides synthesized by other cells in the lung known to express GRP, e.g., pulmonary endocrine cells, are likely to act in a paracrine fashion to stimulate the growth of some non-SCLC tumors expressing bombesin receptors. GRP-R expression is probably important at some stage in the pathogenesis of these particular non-SCLC tumors. Reversal or blockage of these tumors may result upon therapeutic administration of various reagents made available herein.

At least one SCLC line (NCI-N417) reported to show bombesin-dependent growth expressed no detectable mRNA for either GRP-R or NMB-R. This result might be due to the fact that GRP-R and/or NMB-R mRNA is present, but below the level of detection by RNase protection assay. An alternate explanation is that these cells express a bombesin receptor subtype that has not yet been identified. Probes to isolate such receptors are provided herein, and methods for their use are described, e.g., in Example 29.

EXAMPLE 28

Mutagenesis of GRP-R or NMB-R

In vitro or site directed mutagenesis methods are described in standard references, see e.g., Sambrook et al. (1989) or Ausubel et al. (1987 and Supplements), each of which is incorporated herein by reference. Mutagenesis may be directed towards analysis of various different activities and functions of the receptors. In particular, mutagenesis of post-translational modifications sites is of interest to determine, e.g., the effect of glycosylation on various activities. Fusion proteins will be made by standard techniques, typically by recombinant methods. Mutagenesis or replacement of segments homologous to identified phosphorylation sites of other G-protein linked receptors will be performed. Activities of interest include ligand binding, G-protein linkage, phosphorylation activities, and Ca^{++} sequestration. Standard assays for each activity are known and

will be used to specifically identify the structural features which correlate with them.

EXAMPLE 29

Isolation of Homologous Receptors

The present invention provides at least four full length probes for additional receptors for bombesin-like peptides. In particular, genes for a mouse GRP receptor, a rat neuromedin B receptor, and human GRP and NMB receptors are provided. These nucleic acids, or fragments thereof, can be used alone or in combination to screen other DNA sources for sequences having various levels of homology. In particular, the third transmembrane segment has shown high homology among the various receptors for bombesin-like peptides, but other fragments may also be used. Low stringency hybridization of GRP-R and NMB-R probes to Eco RI digested human genomic DNA shows at least six novel fragments which hybridize to either or both probes, but are not the earlier identified human GRP-R or NMB-R gene. See Figure 20. These fragments likely encode exons of additional receptor subtypes for bombesin-like peptides. Genomic cloning, sequencing, and analysis of expression, as applied above, will establish the nature of these hybridizing fragments.

Fifteen micrograms of human genomic DNA were cut with Eco RI, and the fragments resolved by electrophoresis and capillary transferred to nitrocellulose. The nitrocellulose filter was hybridized to a mouse GRP-R cDNA probe (comprising the entire open reading frame of the cDNA) labeled by nick translation to a specific activity of about 300 cpm/pg. Hybridization buffer was 40% formamide, 5X SSC, 20 mM TRIS, 1 X Denhart's solution, 20 micrograms per ml denatured salmon sperm DNA, 10^6 cpm/ml denatured labeled probe. The hybridization was incubated overnight at 37° C. The filter was washed twice in 2 X SSC, 0.1% SDS at room temperature, and twice for fifteen minutes in 0.1 X SSC, 0.1% SDS at 37° C. The blot was exposed to XAR-5 film for several days. Six novel bands are detected, see Figure 20.

Based upon the positive hybridization results on the filter, conditions for a library screen were determined and clones isolated. The sequence of one isolated clone is presented in Table 12. The nucleotide sequence is entered as SEQ ID NO: 9 and the corresponding amino acid sequence is SEQ ID NO: 10. This receptor gene sequence has about 60% nucleotide homology with human R1BP, and its corresponding amino acid sequence has about 50% amino acid identity. Table 13 presents an amino acid sequence comparison between the two.

Table 12: A nucleotide sequence of a human R3BP. The putative coding region has an initiation codon which begins at nucleotide 172 and a termination codon UGA which ends at nucleotide 1371.

```
   1 GAAACACAGA ACTGAAGCAA AGGAGTATCT GGATGTCTTG GATTTTCTTC
  51 CCATTCTGTT CTGTTCTGTT CTCCTAATAC CATCTCGTTA CTAGACGTAG
 101 GCATTGGACG TGACAATCAA CTGCATTTGA ACTGAGAAGA AGAAATATTA
 151 AAGACACAGT CTTCAGAAGA AATGGCTCAA AGGCAGCCTC ACTCACCTAA
 201 TCAGACTTTA ATTTCAATCA CAAATGACAC AGAATCATCA AGCTCTATGG
 251 TTTCTAACGA TAACACAAAT AAAGGATGGA GCGGGGACAA CTCTCCAGGA
 301 ATAGAAGCAT TGTGTGCCAT CTATATTACT TATGCTGTGA TCATTTCAGT
 351 GGGCATCCTT GGAAATGCTA TTCTCATCAA AGTCTTTTTC AAGACCAAAT
 401 CCATGCAAAC AGTTCCAAAT ATTTTCATCA CCAGCCTGGC TTTTGGAGAT
 451 CTTTTACTTC TGCTAACTTG TGTGCCAGTG GATGCAACTC ACTACCTTGC
 501 AGAAGGATGG CTGTTCGGAA GAATTGGTTG TAAGGTGCTC TCTTTCATCC
 551 GGCTCACTTC TGTTGGTGTG TCAGTGTTCA CATTAACAAT TCTCAGCGCT
 601 GACAGATACA AGGCAGTTGT GAAGCCACTT GAGCGACAGC CCTCCAATGC
 651 CATCCTGAAG ACTTGTGTAA AAGCTGGCTG CGTCTGGATC GTGTCTATGA
 701 TATTTGCTCT ACCTGAGGCT ATATTTTCAA ATGTATACAC TTTTCGAGAT
 751 CCCAATAAAA ATATGACATT TGAATCATGT ACCTCTTATC CTGTCTCTAA
 801 GAAGCTCTTG CAAGAAATAC ATTCTCTGCT GTGCTTCTTA GTGTTCTACA
 851 TTATTCCACT CTCTATTATC TCTGTCTACT ATTCCTTGAT TGCTAGGACC
 901 CTTTACAAAA GCACCCTGAA CATACCTACT GAGGAACAAA GCCATGCCCG
 951 TAAGCAGATT GAATCCCGAA AGAGAATTGC CAGAACGGTA TTGGTGTTGG
```

1001 TGGCTCTGTT TGCCCTCTGC TGGTTGCCAA ATCACCTCCT GTACCTCTAC

1051 CATTCATTCA CTTCTCAAAC CTATGTAGAC CCCTCTGCCA TGCATTTCAT

1101 TTTCACCATT TTCTCTCGGG TTTTGGCTTT CAGCAATTCT TGCGTAAACC

1151 CCTTTGCTCT CTACTGGCTG AGCAAAAGCT TCCAGAAGCA TTTTAAAGCT

1201 CAGTTGTTCT GTTGCAAGGC GGAGCGGCCT GAGCCTCCTG TTGCTGACAC

1251 CTCTCTTACC ACCCTGGCTG TGATGGGAAC GGTCCCGGGC ACTGGGAGCA

1301 TACAGATGTC TGAAATTAGT GTGACCTCGT TCACTGGGTG TAGTGTGAAG

1351 CAGGCAGAGG ACAGATTCTA GCTTTTCAAG GAAAAATGCT GCTTCTCCTC

1401 CCAGCGTGTG TATCCGACTC TAAGCTGTGT GCAGGTGTAT GGTGTCCAGA

1451 TTTTTGTTGT TTGAAAAGTG TGTTGAAATC TTAGGAGTGA AGGATCCCTA

1501 TAAGTAAGTA AAATACAAAC CATTACTTTC TTCAAAGTAC AAATAGTAAT

1551 GTCATCGGCT TCTAATAAAT GAGCCCACTA GTGCAGAAAG ACAGTTTATA

1601 TATGCC

Table 13: A comparison of amino acid sequences of human R3BP and human R1BP (GRP-R). The R3BP is above, R1BP is below.

```
  1 MAQRQPHSPNQTLISITNDTESSSSMVSNDNTNKGWSGDNSPGIEALCAI  50
    ||  .:.   | .:  : : . || |  .:  .|.:||    |||  |:.|
  1 MALNDCFLLNLEVDHFMHCNISSHS..ADLPVNDDWS...HPGI..LYVI  43

 51 YITYAVIISVGILGNAILIKVFFKTKSMQTVPNIFITSLAFGDLLLLLTC 100
      .|:||| :|::|| .|||:| ..|||..|||:||.|||:|||||||:||
 44 PAVYGVIILIGLIGNITLIKIFCTVKSMRNVPNLFISSLALGDLLLLITC  93

101 VPVDATHYLAEGWLFGRIGCKVLSFIRLTSVGVSVFTLTILSADRYKAVV 150
    .||||.:|||: |||||||||:|.||.|||||||||||| |||||||||:|
 94 APVDASRYLADRWLFGRIGCKLIPFIQLTSVGVSVFTLTALSADRYKAIV 143

151 KPLERQPSNAILKTCVKAGCVWIVSMIFALPEAIFSNVYTFRDPNKNMTF 200
    :|:: |:|:|::|.|:||: :|||:||::|:|||:||:|..|:|:...| ||
144 RPMDIQASHALMKICLKAAFIWIISMLLAIPEAVFSDLHPFHEESTNQTF 193

201 ESCTSYPVSKKLLQEIHSLLCFLVFYIIPLSIISVYYSLIARTLYKSTLN 250
     ||..|| |..| ..|||: :|||||:|||||||||| :||:.|..|..|
194 ISCAPYPHSNELHPKIHSMASFLVFYVIPLSIISVYYYFIAKNLIQSAYN 243

251 IPTEEQSHARKQIESRKRIARTVLVLVALFALCWLPNHLLYLYHSFTSQT 300
    :|.|:. |.:|||||||:|:||||:|:|||:||||||::|||:|:    .
244 LPVEGNIHVKKQIESRKRLAKTVLVFVGLFAFCWLPNHVIYLYRSYHYSE 293

301 YVDPSAMHFIFTIFSRVLAFSNSCVNPFALYWLSKSFQKHFKAQLFCCKA 350
     ||.| :||: .| .|:|||.||||||||||:|||||.|:|..||:||.:
294 .VDTSMLHFVTSICARLLAFTNSCVNPFALYLLSKSFRKQFNTQLLCCQP 342

351 ERPEPPVADTSLTTLAVMGTVPGTGSIQMSEISVTSFTGCSVKQAEDRF* 400
    :   .. .... ||        . .|:. .: ||..|. .... ..:|:
343 GLIIRSHSTGRSTT.......CMTSLKSTNPSVATFSLINGNICHERY. 383
```

Amplification methods, e.g., polymerase chain reaction techniques, may also be used with these probes to isolate and purify additional receptors.

Alternatively, other screening methods using antibodies or activity assays will be used to verify or assist in the isolation of new receptors. Expression of receptor may be screened by antibodies or endocrine stimulation of cells expressing the appropriate receptor sequences.

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

Table 14: In the claims, the following SEQ ID NO: correspondences are intended:

SEQ ID NO:	corresponds to	which is
1	Table 1	mouse R1BP (GRP-R) nucleic acid
2	Table 1	mouse R1BP (GRP-R) amino acid
3	Table 2	human R1BP (GRP-R) nucleic acid
4	Table 2	human R1BP (GRP-R) amino acid
5	Table 3	rat R2BP (NMB-R) nucleic acid
6	Table 3	rat R2BP (NMB-R) amino acid
7	Table 4	human R2BP (NMB-R) nucleic acid
8	Table 4	human R2BP (NMB-R) amino acid
9	Table 12	human R3BP nucleic acid
10		human R3BP amino acid

SEQUENCE LISTING

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(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/426,150
(B) FILING DATE: 24-OCT-1989

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/533,659
(B) FILING DATE: 05-JUN-1990

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1700 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus
(H) CELL LINE: Swiss 3T3

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Lambda GT10

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 378..1532

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
AAAACTGCAG CCAGAGAGAC TCAGTCTAGG ATGGAGGTAG GAAGAGCTGA GACAAAGTGG      60

GCTTAATTCT AAGCTTTTCT TCAGGCTGAG TTTCTGTTGC TTGTTAACTT AGTGAATGTA     120

CAGATGTATT GCTTGCTGGT GGTGTGAAGG CTGGGACAGA ACCAACATCA ACAAACTGAG     180

CTAGAGTTTG GAATACCAGT GTGTGTGTGT GTGTGTGTGT GTGTGTGTGT GTGTGTGAAT     240

TCAGAGTGTT TTAAAGAGAG ATCAAGAGGC TCACACAGAT CAGCGAGCCT AACTGACAAA     300

CCTTCAGCGC CTAACTGAAA AACCCAGAAG TTACAAAGCA GCATCTTGAA GGCGCATTTG     360

AAGAGAGAAG CTTTGAG ATG GCT CCA AAT AAT TGT TCC CAC CTG AAC TTG         410
                  Met Ala Pro Asn Asn Cys Ser His Leu Asn Leu
                    1               5                  10

GAC GTG GAC CCT TTC CTG TCC TGC AAC GAC ACC TTC AAT CAA AGT CTG       458
Asp Val Asp Pro Phe Leu Ser Cys Asn Asp Thr Phe Asn Gln Ser Leu
            15                  20                  25

AGT CCC CCC AAG ATG GAC AAC TGG TTT CAC CCG GGC TTC ATC TAT GTC       506
Ser Pro Pro Lys Met Asp Asn Trp Phe His Pro Gly Phe Ile Tyr Val
        30                  35                  40

ATC CCT GCA GTT TAT GGG CTT ATC ATC GTG ATA GGT CTT ATT GGC AAC       554
Ile Pro Ala Val Tyr Gly Leu Ile Ile Val Ile Gly Leu Ile Gly Asn
    45                  50                  55
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ATC ACG CTC ATC AAG ATC TTC TGC ACG GTC AAG TCC ATG CGA AAC GTG                602
Ile Thr Leu Ile Lys Ile Phe Cys Thr Val Lys Ser Met Arg Asn Val
 60                  65                  70                  75

CCA AAC CTG TTC ATC TCT AGC CTG GCT TTG GGA GAC CTG CTG CTG CTG                650
Pro Asn Leu Phe Ile Ser Ser Leu Ala Leu Gly Asp Leu Leu Leu Leu
                 80                  85                  90

GTG ACA TGC GCC CCT GTG GAT GCC AGC AAG TAC CTG GCT GAC AGG TGG                698
Val Thr Cys Ala Pro Val Asp Ala Ser Lys Tyr Leu Ala Asp Arg Trp
            95                 100                 105

CTA TTT GGC AGA ATT GGC TGC AAA CTG ATC CCC TTT ATA CAA CTT ACT                746
Leu Phe Gly Arg Ile Gly Cys Lys Leu Ile Pro Phe Ile Gln Leu Thr
        110                 115                 120

TCA GTG GGG GTG TCT GTC TTC ACA CTT ACG GCA CTG TCA GCT GAC AGG                794
Ser Val Gly Val Ser Val Phe Thr Leu Thr Ala Leu Ser Ala Asp Arg
    125                 130                 135

TAC AAA GCC ATT GTA CGG CCA ATG GAT ATC CAG GCA TCC CAT GCC CTG                842
Tyr Lys Ala Ile Val Arg Pro Met Asp Ile Gln Ala Ser His Ala Leu
140                 145                 150                 155

ATG AAG ATC TGT CTC AAA GCT GCT TTG ATC TGG ATT GTC TCT ATG TTG                890
Met Lys Ile Cys Leu Lys Ala Ala Leu Ile Trp Ile Val Ser Met Leu
                160                 165                 170

TTG GCC ATC CCA GAG GCT GTG TTT TCT GAC CTC CAC CCC TTC CAT GTG                938
Leu Ala Ile Pro Glu Ala Val Phe Ser Asp Leu His Pro Phe His Val
            175                 180                 185

AAA GAT ACC AAC CAA ACC TTC ATT AGT TGT GCC CCC TAC CCA CAC TCC                986
Lys Asp Thr Asn Gln Thr Phe Ile Ser Cys Ala Pro Tyr Pro His Ser
        190                 195                 200

AAT GAG CTA CAC CCT AAA ATC CAT TCC ATG GCT TCC TTT CTG GTT TTC               1034
Asn Glu Leu His Pro Lys Ile His Ser Met Ala Ser Phe Leu Val Phe
    205                 210                 215

TAC GTT ATC CCA CTG GCG ATC ATC TCT GTC TAC TAC TAC TTC ATT GCC               1082
Tyr Val Ile Pro Leu Ala Ile Ile Ser Val Tyr Tyr Tyr Phe Ile Ala
220                 225                 230                 235

CGA AAT CTG ATT CAG AGT GCC TAC AAT CTT CCC GTG GAA GGC AAT ATA               1130
Arg Asn Leu Ile Gln Ser Ala Tyr Asn Leu Pro Val Glu Gly Asn Ile
                240                 245                 250

CAT GTC AAG AAG CAG ATC GAA TCC CGG AAG CGG CTT GCC AAG ACA GTA               1178
His Val Lys Lys Gln Ile Glu Ser Arg Lys Arg Leu Ala Lys Thr Val
            255                 260                 265

CTG GTG TTT GTG GGC CTC TTT GCC TTC TGC TGG CTC CCC AAC CAT GTC               1226
Leu Val Phe Val Gly Leu Phe Ala Phe Cys Trp Leu Pro Asn His Val
        270                 275                 280
```

```
ATC TAC CTG TAC CGT TCC TAC CAC TAC TCT GAA GTG GAC ACC TCC ATG      1274
Ile Tyr Leu Tyr Arg Ser Tyr His Tyr Ser Glu Val Asp Thr Ser Met
    285                 290                 295

CTC CAC TTT GTC ACC AGC ATC TGT GCC CAC CTC CTG GCC TTC ACC AAC      1322
Leu His Phe Val Thr Ser Ile Cys Ala His Leu Leu Ala Phe Thr Asn
300                 305                 310                 315

TCC TGT GTG AAC CCC TTT GCT CTT TAT CTG CTG AGC AAG AGC TTC AGG      1370
Ser Cys Val Asn Pro Phe Ala Leu Tyr Leu Leu Ser Lys Ser Phe Arg
                320                 325                 330

AAG CAG TTC AAC ACT CAA CTT CTC TGC TGC CAG CCT GGC CTG ATG AAC      1418
Lys Gln Phe Asn Thr Gln Leu Leu Cys Cys Gln Pro Gly Leu Met Asn
            335                 340                 345

AGG TCC CAC AGC ACA GGC AGA AGT ACC ACC TGC ATG ACC TCC TTC AAG      1466
Arg Ser His Ser Thr Gly Arg Ser Thr Thr Cys Met Thr Ser Phe Lys
        350                 355                 360

AGC ACT AAC CCC TCG GCT ACC TTT AGC CTC ATC AAC AGA AAT ATC TGT      1514
Ser Thr Asn Pro Ser Ala Thr Phe Ser Leu Ile Asn Arg Asn Ile Cys
    365                 370                 375

CAT GAG GGG TAT GTC TAGACTAAAC TTCAACCTTG CCTCTAAAGG AACTCCTGGT      1569
His Glu Gly Tyr Val
380                 385

ATTGTTCTAC AGATGTCCAG GGGCCCTGAG ATTGATTGTT GTCTCTATAT CTTCTGAAGA    1629

CTCTTCAGGG GGATGAGTGA TACAGACGGA TGGGAAAGAT GTCCAAATGC ACCAATCACC    1689

ATTGTATCTC A                                                        1700
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 384 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ala Pro Asn Asn Cys Ser His Leu Asn Leu Asp Val Asp Pro Phe
  1               5                  10                  15

Leu Ser Cys Asn Asp Thr Phe Asn Gln Ser Leu Ser Pro Pro Lys Met
            20                  25                  30

Asp Asn Trp Phe His Pro Gly Phe Ile Tyr Val Ile Pro Ala Val Tyr
        35                  40                  45
```

Gly Leu Ile Ile Val Ile Gly Leu Ile Gly Asn Ile Thr Leu Ile Lys
50 55 60

Ile Phe Cys Thr Val Lys Ser Met Arg Asn Val Pro Asn Leu Phe Ile
65 70 75 80

Ser Ser Leu Ala Leu Gly Asp Leu Leu Leu Leu Val Thr Cys Ala Pro
85 90 95

Val Asp Ala Ser Lys Tyr Leu Ala Asp Arg Trp Leu Phe Gly Arg Ile
100 105 110

Gly Cys Lys Leu Ile Pro Phe Ile Gln Leu Thr Ser Val Gly Val Ser
115 120 125

Val Phe Thr Leu Thr Ala Leu Ser Ala Asp Arg Tyr Lys Ala Ile Val
130 135 140

Arg Pro Met Asp Ile Gln Ala Ser His Ala Leu Met Lys Ile Cys Leu
145 150 155 160

Lys Ala Ala Leu Ile Trp Ile Val Ser Met Leu Leu Ala Ile Pro Glu
165 170 175

Ala Val Phe Ser Asp Leu His Pro Phe His Val Lys Asp Thr Asn Gln
180 185 190

Thr Phe Ile Ser Cys Ala Pro Tyr Pro His Ser Asn Glu Leu His Pro
195 200 205

Lys Ile His Ser Met Ala Ser Phe Leu Val Phe Tyr Val Ile Pro Leu
210 215 220

Ala Ile Ile Ser Val Tyr Tyr Tyr Phe Ile Ala Arg Asn Leu Ile Gln
225 230 235 240

Ser Ala Tyr Asn Leu Pro Val Glu Gly Asn Ile His Val Lys Lys Gln
245 250 255

Ile Glu Ser Arg Lys Arg Leu Ala Lys Thr Val Leu Val Phe Val Gly
260 265 270

Leu Phe Ala Phe Cys Trp Leu Pro Asn His Val Ile Tyr Leu Tyr Arg
275 280 285

Ser Tyr His Tyr Ser Glu Val Asp Thr Ser Met Leu His Phe Val Thr
290 295 300

Ser Ile Cys Ala His Leu Leu Ala Phe Thr Asn Ser Cys Val Asn Pro
305 310 315 320

Phe Ala Leu Tyr Leu Leu Ser Lys Ser Phe Arg Lys Gln Phe Asn Thr
325 330 335

Gln Leu Leu Cys Cys Gln Pro Gly Leu Met Asn Arg Ser His Ser Thr
340 345 350

Gly Arg Ser Thr Thr Cys Met Thr Ser Phe Lys Ser Thr Asn Pro Ser
355 360 365

Ala Thr Phe Ser Leu Ile Asn Arg Asn Ile Cys His Glu Gly Tyr Val
370 375 380

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1726 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(G) CELL TYPE: Small cell lung carcinoma
(H) CELL LINE: NCI-H345

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Lambda GT10

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 399..1553

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCAGATTCTA AATATCAGGA AAGACGCTGT GGGAAAATAG CAGGCCAAAA GTTCTTAGTA 60

AACTGCAGCC AGGGAGACTC AGACTAGAAT GGAGGTAGAA AGAACTGATG CAGAGTGGGT 120

TTAATTCTAA GCCTTTTTGT GGCTAAGTTT TGTTGTTGTT AACTTATTGA ATTTAGAGTT 180

GTATTGCACT GGTCATGTGA AAGCCAGAGC AGCACCAGTG TCAAAATAGT GACAGAGAGT 240

TTTGAATACC ATAGTTAGTA TATATGTACT CAGAGTATTT TTATTAAAGA AGGCAAAGAG 300

CCCGGCATAG ATCTTATCTT CATCTTCACT CGGTTGCAAA ATCAATAGTT AAGAAATAGC 360

ATCTAAGGGA ACTTTTAGGT GGGAAAAAAA ATCTAGAG ATG GCT CTA AAT GAC 413
Met Ala Leu Asn Asp
1 5

TGT TTC CTT CTG AAC TTG GAG GTG GAC CAT TTC ATG CAC TGC AAC ATC 461
Cys Phe Leu Leu Asn Leu Glu Val Asp His Phe Met His Cys Asn Ile
10 15 20

```
TCC AGT CAC AGT GCG GAT CTC CCC GTG AAC GAT GAC TGG TCC CAC CCG          509
Ser Ser His Ser Ala Asp Leu Pro Val Asn Asp Asp Trp Ser His Pro
              25                  30                  35

GGG ATC CTC TAT GTC ATC CCT GCA GTT TAT GGG GTT ATC ATT CTG ATA          557
Gly Ile Leu Tyr Val Ile Pro Ala Val Tyr Gly Val Ile Ile Leu Ile
          40                  45                  50

GGC CTC ATT GGC AAC ATC ACT TTG ATC AAG ATC TTC TGT ACA GTC AAG          605
Gly Leu Ile Gly Asn Ile Thr Leu Ile Lys Ile Phe Cys Thr Val Lys
     55                  60                  65

TCC ATG CGA AAC GTT CCA AAC CTG TTC ATT TCC AGT CTG GCT TTG GGA          653
Ser Met Arg Asn Val Pro Asn Leu Phe Ile Ser Ser Leu Ala Leu Gly
 70                  75                  80                  85

GAC CTG CTC CTC CTA ATA ACG TGT GCT CCA GTG GAT GCC AGC AGG TAC          701
Asp Leu Leu Leu Leu Ile Thr Cys Ala Pro Val Asp Ala Ser Arg Tyr
                 90                  95                 100

CTG GCT GAC AGA TGG CTA TTT GGC AGG ATT GGC TGC AAA CTG ATC CCC          749
Leu Ala Asp Arg Trp Leu Phe Gly Arg Ile Gly Cys Lys Leu Ile Pro
             105                 110                 115

TTT ATA CAG CTT ACC TCT GTT GGG GTG TCT GTC TTC ACA CTC ACG GCG          797
Phe Ile Gln Leu Thr Ser Val Gly Val Ser Val Phe Thr Leu Thr Ala
         120                 125                 130

CTC TCG GCA GAC AGA TAC AAA GCC ATT GTC CGG CCA ATG GAT ATC CAG          845
Leu Ser Ala Asp Arg Tyr Lys Ala Ile Val Arg Pro Met Asp Ile Gln
     135                 140                 145

GCC TCC CAT GCC CTG ATG AAG ATC TGC CTC AAA GCC GCC TTT ATC TGG          893
Ala Ser His Ala Leu Met Lys Ile Cys Leu Lys Ala Ala Phe Ile Trp
 150                 155                 160                 165

ATC ATC TCC ATG CTG CTG GCC ATT CCA GAG GCC GTG TTT TCT GAC CTC          941
Ile Ile Ser Met Leu Leu Ala Ile Pro Glu Ala Val Phe Ser Asp Leu
                 170                 175                 180

CAT CCC TTC CAT GAG GAA AGC ACC AAC CAG ACC TTC ATT AGC TGT GCC          989
His Pro Phe His Glu Glu Ser Thr Asn Gln Thr Phe Ile Ser Cys Ala
             185                 190                 195

CCA TAC CCA CAC TCT AAT GAG CTT CAC CCC AAA ATC CAT TCT ATG GCT         1037
Pro Tyr Pro His Ser Asn Glu Leu His Pro Lys Ile His Ser Met Ala
         200                 205                 210

TCC TTT CTG GTC TTC TAC GTC ATC CCA CTG TCG ATC ATC TCT GTT TAC         1085
Ser Phe Leu Val Phe Tyr Val Ile Pro Leu Ser Ile Ile Ser Val Tyr
     215                 220                 225

TAC TAC TTC ATT GCT AAA AAT CTG ATC CAG AGT GCT TAC AAT CTT CCC         1133
Tyr Tyr Phe Ile Ala Lys Asn Leu Ile Gln Ser Ala Tyr Asn Leu Pro
 230                 235                 240                 245
```

```
GTG GAA GGG AAT ATA CAT GTC AAG AAG CAG ATT GAA TCC CGG AAG CGA      1181
Val Glu Gly Asn Ile His Val Lys Lys Gln Ile Glu Ser Arg Lys Arg
                250                 255                 260

CTT GCC AAG ACA GTG CTG GTG TTT GTG GGC CTG TTC GCC TTC TGC TGG      1229
Leu Ala Lys Thr Val Leu Val Phe Val Gly Leu Phe Ala Phe Cys Trp
            265                 270                 275

CTC CCC AAT CAT GTC ATC TAC CTG TAC CGC TCC TAC CAC TAC TCT GAG      1277
Leu Pro Asn His Val Ile Tyr Leu Tyr Arg Ser Tyr His Tyr Ser Glu
        280                 285                 290

GTG GAC ACC TCC ATG CTC CAC TTT GTC ACC AGC ATC TGT GCC CGC CTC      1325
Val Asp Thr Ser Met Leu His Phe Val Thr Ser Ile Cys Ala Arg Leu
    295                 300                 305

CTG GCC TTC ACC AAC TCC TGC GTG AAC CCC TTT GCC CTC TAC CTG CTG      1373
Leu Ala Phe Thr Asn Ser Cys Val Asn Pro Phe Ala Leu Tyr Leu Leu
310                 315                 320                 325

AGC AAG AGT TTC AGG AAA CAG TTC AAC ACT CAG CTG CTC TGT TGC CAG      1421
Ser Lys Ser Phe Arg Lys Gln Phe Asn Thr Gln Leu Leu Cys Cys Gln
                330                 335                 340

CCT GGC CTG ATC ATC CGG TCT CAC AGC ACT GGA AGG AGT ACA ACC TGC      1469
Pro Gly Leu Ile Ile Arg Ser His Ser Thr Gly Arg Ser Thr Thr Cys
            345                 350                 355

ATG ACC TCC CTC AAG AGT ACC AAC CCC TCC GTG GCC ACC TTT AGC CTC      1517
Met Thr Ser Leu Lys Ser Thr Asn Pro Ser Val Ala Thr Phe Ser Leu
        360                 365                 370

ATC AAT GGA AAC ATC TGT CAC GAG CGG TAT GTC TAGATTGACC CTTGATTTTG    1570
Ile Asn Gly Asn Ile Cys His Glu Arg Tyr Val
    375                 380                 385

CCCCCTGAGG GACGGTTTTG CTTTATGGCT AGACAGGAAC CCTTGCATCC ATTGTTGTGT    1630

CTGTGCCCTC CAAAGAGCCT TCAGAATGCT CCTGAGTGGT GTAGGTGGGG GTGGGGAGGC    1690

CCAAATGATG GATCACCATT ATATTTTGAA AGAAGC                              1726
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 384 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Leu Asn Asp Cys Phe Leu Leu Asn Leu Glu Val Asp His Phe
 1               5                  10                  15
```

Met His Cys Asn Ile Ser Ser His Ser Ala Asp Leu Pro Val Asn Asp
20 25 30

Asp Trp Ser His Pro Gly Ile Leu Tyr Val Ile Pro Ala Val Tyr Gly
35 40 45

Val Ile Ile Leu Ile Gly Leu Ile Gly Asn Ile Thr Leu Ile Lys Ile
50 55 60

Phe Cys Thr Val Lys Ser Met Arg Asn Val Pro Asn Leu Phe Ile Ser
65 70 75 80

Ser Leu Ala Leu Gly Asp Leu Leu Leu Leu Ile Thr Cys Ala Pro Val
85 90 95

Asp Ala Ser Arg Tyr Leu Ala Asp Arg Trp Leu Phe Gly Arg Ile Gly
100 105 110

Cys Lys Leu Ile Pro Phe Ile Gln Leu Thr Ser Val Gly Val Ser Val
115 120 125

Phe Thr Leu Thr Ala Leu Ser Ala Asp Arg Tyr Lys Ala Ile Val Arg
130 135 140

Pro Met Asp Ile Gln Ala Ser His Ala Leu Met Lys Ile Cys Leu Lys
145 150 155 160

Ala Ala Phe Ile Trp Ile Ile Ser Met Leu Leu Ala Ile Pro Glu Ala
165 170 175

Val Phe Ser Asp Leu His Pro Phe His Glu Glu Ser Thr Asn Gln Thr
180 185 190

Phe Ile Ser Cys Ala Pro Tyr Pro His Ser Asn Glu Leu His Pro Lys
195 200 205

Ile His Ser Met Ala Ser Phe Leu Val Phe Tyr Val Ile Pro Leu Ser
210 215 220

Ile Ile Ser Val Tyr Tyr Tyr Phe Ile Ala Lys Asn Leu Ile Gln Ser
225 230 235 240

Ala Tyr Asn Leu Pro Val Glu Gly Asn Ile His Val Lys Lys Gln Ile
245 250 255

Glu Ser Arg Lys Arg Leu Ala Lys Thr Val Leu Val Phe Val Gly Leu
260 265 270

Phe Ala Phe Cys Trp Leu Pro Asn His Val Ile Tyr Leu Tyr Arg Ser
275 280 285

Tyr His Tyr Ser Glu Val Asp Thr Ser Met Leu His Phe Val Thr Ser
290 295 300

Ile Cys Ala Arg Leu Leu Ala Phe Thr Asn Ser Cys Val Asn Pro Phe
305 310 315 320

Ala Leu Tyr Leu Leu Ser Lys Ser Phe Arg Lys Gln Phe Asn Thr Gln
325 330 335

Leu Leu Cys Cys Gln Pro Gly Leu Ile Ile Arg Ser His Ser Thr Gly
340 345 350

Arg Ser Thr Thr Cys Met Thr Ser Leu Lys Ser Thr Asn Pro Ser Val
355 360 365

Ala Thr Phe Ser Leu Ile Asn Gly Asn Ile Cys His Glu Arg Tyr Val
370 375 380

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1584 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rattus rattus
(F) TISSUE TYPE: Esophagus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 132..1304

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGTGGCTCAG TTCCAGGAGC CACAAACTTG CCAGGATCAG AGACAATCAA CTAAACCCAG 60

GTCGTACTCA CCGCACTTTC GAGACGCGCG AGTGCAGGAA AACTCCCGCG AATCCCCTGG 120

GAAAGGACAC C ATG CCC CCC AGG TCT CTC CCC AAC CTC TCC TTG CCC ACC 170
Met Pro Pro Arg Ser Leu Pro Asn Leu Ser Leu Pro Thr
1 5 10

GAG GCG AGC GAG AGC GAG TTG GAA CCC GAG GTG TGG GAA AAT GAT TTC 218
Glu Ala Ser Glu Ser Glu Leu Glu Pro Glu Val Trp Glu Asn Asp Phe
15 20 25

CTG CCT GAC TCA GAC GGG ACC ACC GCG GAG TTG GTA ATC CGC TGT GTG 266
Leu Pro Asp Ser Asp Gly Thr Thr Ala Glu Leu Val Ile Arg Cys Val
30 35 40 45

ATA CCA TCC CTC TAC CTA ATC ATC ATC TCG GTG GGC TTG CTG GGC AAC 314
Ile Pro Ser Leu Tyr Leu Ile Ile Ile Ser Val Gly Leu Leu Gly Asn
50 55 60

```
ATC ATG CTG GTG AAG ATA TTC CTC ACC AAC AGC ACC ATG CGG AGT GTC          362
Ile Met Leu Val Lys Ile Phe Leu Thr Asn Ser Thr Met Arg Ser Val
            65                  70                  75

CCC AAC ATC TTC ATC TCT AAC CTG GCT GCG GGA GAC CTG CTG CTG CTG          410
Pro Asn Ile Phe Ile Ser Asn Leu Ala Ala Gly Asp Leu Leu Leu Leu
        80                  85                  90

CTG ACC TGC GTC CCA GTG GAT GCC TCC CGA TAC TTC TTT GAT GAA TGG          458
Leu Thr Cys Val Pro Val Asp Ala Ser Arg Tyr Phe Phe Asp Glu Trp
    95                  100                 105

GTG TTC GGC AAG CTG GGC TGC AAA CTC ATC CCA GCC ATC CAG CTC ACC          506
Val Phe Gly Lys Leu Gly Cys Lys Leu Ile Pro Ala Ile Gln Leu Thr
110                 115                 120                 125

TCG GTG GGG GTT TCC GTG TTC ACT CTC ACG GCC CTC AGC GCT GAC AGG          554
Ser Val Gly Val Ser Val Phe Thr Leu Thr Ala Leu Ser Ala Asp Arg
                130                 135                 140

TAC AGA GCT ATC GTG AAC CCC ATG GAC ATG CAG ACG TCT GGT GTG GTG          602
Tyr Arg Ala Ile Val Asn Pro Met Asp Met Gln Thr Ser Gly Val Val
            145                 150                 155

CTG TGG ACC AGT TTG AAG GCC GTG GGC ATC TGG GTG GTC TCT GTG CTG          650
Leu Trp Thr Ser Leu Lys Ala Val Gly Ile Trp Val Val Ser Val Leu
        160                 165                 170

TTG GCT GTC CCT GAG GCT GTG TTT TCG GAA GTA GCA CGC ATC GGT AGC          698
Leu Ala Val Pro Glu Ala Val Phe Ser Glu Val Ala Arg Ile Gly Ser
    175                 180                 185

TCG GAT AAC AGC AGT TTC ACA GCA TGC ATA CCC TAC CCA CAA ACA GAT          746
Ser Asp Asn Ser Ser Phe Thr Ala Cys Ile Pro Tyr Pro Gln Thr Asp
190                 195                 200                 205

GAG TTA CAT CCA AAG ATC CAC TCA GTG CTC ATT TTT CTT GTC TAT TTC          794
Glu Leu His Pro Lys Ile His Ser Val Leu Ile Phe Leu Val Tyr Phe
                210                 215                 220

CTC ATA CCC CTT GTT ATC ATC AGC ATT TAT TAT TAT CAC ATT GCG AAG          842
Leu Ile Pro Leu Val Ile Ile Ser Ile Tyr Tyr Tyr His Ile Ala Lys
            225                 230                 235

ACT TTA ATT AGA AGT GCA CAC AAT CTT CCT GGA GAA TAC AAT GAA CAT          890
Thr Leu Ile Arg Ser Ala His Asn Leu Pro Gly Glu Tyr Asn Glu His
        240                 245                 250

ACC AAA AAG CAG ATG GAG ACA CGG AAA CGC CTG GCC AAG ATC GTT CTG          938
Thr Lys Lys Gln Met Glu Thr Arg Lys Arg Leu Ala Lys Ile Val Leu
    255                 260                 265

GTG TTT GTG GGC TGC TTT GTC TTC TGC TGG TTT CCC AAC CAC ATC CTC          986
Val Phe Val Gly Cys Phe Val Phe Cys Trp Phe Pro Asn His Ile Leu
270                 275                 280                 285
```

```
TAC TTG TAT AGG TCT TTC AAC TAC AAG GAG ATC GAT CCT TCT CTT GGA      1034
Tyr Leu Tyr Arg Ser Phe Asn Tyr Lys Glu Ile Asp Pro Ser Leu Gly
                290                 295                 300

CAC ATG ATT GTC ACC TTA GTG GCC CGG GTT CTG AGT TTC AGC AAC TCC      1082
His Met Ile Val Thr Leu Val Ala Arg Val Leu Ser Phe Ser Asn Ser
            305                 310                 315

TGT GTC AAC CCG TTT GCT CTT TAC CTG CTC AGT GAA AGC TTC AGG AAG      1130
Cys Val Asn Pro Phe Ala Leu Tyr Leu Leu Ser Glu Ser Phe Arg Lys
        320                 325                 330

CAT TTC AAC AGC CAG CTC TGT TGT GGG CAG AAG TCC TAT CCT GAG AGG      1178
His Phe Asn Ser Gln Leu Cys Cys Gly Gln Lys Ser Tyr Pro Glu Arg
    335                 340                 345

TCT ACC AGC TAC CTC CTC AGC TCT TCA GCA GTA AGA ATG ACT TCT CTG      1226
Ser Thr Ser Tyr Leu Leu Ser Ser Ser Ala Val Arg Met Thr Ser Leu
350                 355                 360                 365

AAA AGC AAC GCG AAG AAT GTG GTG ACC AAT TCT GTC CTG CTC AAC GGA      1274
Lys Ser Asn Ala Lys Asn Val Val Thr Asn Ser Val Leu Leu Asn Gly
                370                 375                 380

CAT AGC ACA AAG CAA GAA ATA GCA CTG TGATCGGAGA CCATCCAATT            1321
His Ser Thr Lys Gln Glu Ile Ala Leu
            385                 390

CATCCTCGGG AAATACCATT TTCACAACTT TTCCATTATT ATTGAGCGAA GCAGAGCTAA    1381

ATAATCACCA CATTTACACT GCTCCCCAGC TAATTCATGA TTGACTCAAG CGCAAGGCAC    1441

GCACTTTTGT CTGAATAGAA AGAATTTTAC CTTACACCAC CACACATCTA ACTCACACGT    1501

AATTCACATA TATCTCCTGC TAACATCGGT TTACACATTC CCTTGGGATT TAAGACATTC    1561

CAACAAGCAA ATGTGGCATA TTG                                            1584
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 390 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Pro Pro Arg Ser Leu Pro Asn Leu Ser Leu Pro Thr Glu Ala Ser
  1               5                  10                  15

Glu Ser Glu Leu Glu Pro Glu Val Trp Glu Asn Asp Phe Leu Pro Asp
            20                  25                  30
```

Ser Asp Gly Thr Thr Ala Glu Leu Val Ile Arg Cys Val Ile Pro Ser
35 40 45

Leu Tyr Leu Ile Ile Ile Ser Val Gly Leu Leu Gly Asn Ile Met Leu
50 55 60

Val Lys Ile Phe Leu Thr Asn Ser Thr Met Arg Ser Val Pro Asn Ile
65 70 75 80

Phe Ile Ser Asn Leu Ala Ala Gly Asp Leu Leu Leu Leu Leu Thr Cys
85 90 95

Val Pro Val Asp Ala Ser Arg Tyr Phe Phe Asp Glu Trp Val Phe Gly
100 105 110

Lys Leu Gly Cys Lys Leu Ile Pro Ala Ile Gln Leu Thr Ser Val Gly
115 120 125

Val Ser Val Phe Thr Leu Thr Ala Leu Ser Ala Asp Arg Tyr Arg Ala
130 135 140

Ile Val Asn Pro Met Asp Met Gln Thr Ser Gly Val Val Leu Trp Thr
145 150 155 160

Ser Leu Lys Ala Val Gly Ile Trp Val Val Ser Val Leu Leu Ala Val
165 170 175

Pro Glu Ala Val Phe Ser Glu Val Ala Arg Ile Gly Ser Ser Asp Asn
180 185 190

Ser Ser Phe Thr Ala Cys Ile Pro Tyr Pro Gln Thr Asp Glu Leu His
195 200 205

Pro Lys Ile His Ser Val Leu Ile Phe Leu Val Tyr Phe Leu Ile Pro
210 215 220

Leu Val Ile Ile Ser Ile Tyr Tyr Tyr His Ile Ala Lys Thr Leu Ile
225 230 235 240

Arg Ser Ala His Asn Leu Pro Gly Glu Tyr Asn Glu His Thr Lys Lys
245 250 255

Gln Met Glu Thr Arg Lys Arg Leu Ala Lys Ile Val Leu Val Phe Val
260 265 270

Gly Cys Phe Val Phe Cys Trp Phe Pro Asn His Ile Leu Tyr Leu Tyr
275 280 285

Arg Ser Phe Asn Tyr Lys Glu Ile Asp Pro Ser Leu Gly His Met Ile
290 295 300

Val Thr Leu Val Ala Arg Val Leu Ser Phe Ser Asn Ser Cys Val Asn
305 310 315 320

Pro Phe Ala Leu Tyr Leu Leu Ser Glu Ser Phe Arg Lys His Phe Asn
325 330 335

Ser Gln Leu Cys Cys Gly Gln Lys Ser Tyr Pro Glu Arg Ser Thr Ser
340 345 350

Tyr Leu Leu Ser Ser Ser Ala Val Arg Met Thr Ser Leu Lys Ser Asn
355 360 365

Ala Lys Asn Val Val Thr Asn Ser Val Leu Leu Asn Gly His Ser Thr
370 375 380

Lys Gln Glu Ile Ala Leu
385 390

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1352 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(G) CELL TYPE: Small cell lung carcinoma
(H) CELL LINE: NCI-H345

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 140..1312

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GTGCTGTGAG GCTTGCCCGC GGACAGTAAA CTTGCAGGGG CGAGAGGGAG GGACATCGAT 60

TAAACCTAAA TCGTGGGCGT TCAGTCCTCA GGGCACCGAG CGCGTGAAAA CTCCAGCGGA 120

CTCTGCTGGA AAGGAGATC ATG CCC TCT AAG TCT CTT TCC AAC CTC TCG GTG 172
Met Pro Ser Lys Ser Leu Ser Asn Leu Ser Val
1 5 10

ACC ACC GGC GCG AAT GAG AGC GGT TCC GTT CCC GAG GGG TGG GAA AGG 220
Thr Thr Gly Ala Asn Glu Ser Gly Ser Val Pro Glu Gly Trp Glu Arg
15 20 25

GAT TTC CTG CCG GCC TCG GAC GGG ACC ACC ACG GAG TTG GTG ATC CGC 268
Asp Phe Leu Pro Ala Ser Asp Gly Thr Thr Thr Glu Leu Val Ile Arg
30 35 40

TGT GTG ATC CCG TCC CTC TAC CTG CTC ATC ATC ACC GTG GGC TTG CTG 316
Cys Val Ile Pro Ser Leu Tyr Leu Leu Ile Ile Thr Val Gly Leu Leu
45 50 55

GGC AAC ATC ATG CTG GTG AAG ATC TTC ATC ACC AAC AGC GCC ATG AGG 364
Gly Asn Ile Met Leu Val Lys Ile Phe Ile Thr Asn Ser Ala Met Arg
60 65 70 75

AGC GTC CCC AAC ATC TTC ATC TCT AAC CTG GCG GCC GGG GAC TTG CTG 412
Ser Val Pro Asn Ile Phe Ile Ser Asn Leu Ala Ala Gly Asp Leu Leu
80 85 90

CTG CTG CTC ACC TGC GTC CCG GTG GAC GCC TCG CGC TAC TTC TTC GAC 460
Leu Leu Leu Thr Cys Val Pro Val Asp Ala Ser Arg Tyr Phe Phe Asp
95 100 105

GAG TGG ATG TTT GGC AAG GTG GGC TGC AAA CTG ATC CCT GTC ATC CAG 508
Glu Trp Met Phe Gly Lys Val Gly Cys Lys Leu Ile Pro Val Ile Gln
110 115 120

CTC ACT TCC GTG GGG GTT TCC GTG TTC ACT CTC ACT GCC CTC AGC GCC 556
Leu Thr Ser Val Gly Val Ser Val Phe Thr Leu Thr Ala Leu Ser Ala
125 130 135

GAC AGG TAC AGA GCC ATC GTT AAC CCC ATG GAC ATG CAG ACG TCA GGG 604
Asp Arg Tyr Arg Ala Ile Val Asn Pro Met Asp Met Gln Thr Ser Gly
140 145 150 155

GCA TTG CTG CGG ACC TGT GTG AAG GCC ATG GGT ATC TGG GTG GTC TCC 652
Ala Leu Leu Arg Thr Cys Val Lys Ala Met Gly Ile Trp Val Val Ser
160 165 170

GTG TTG CTG GCA GTT CCC GAA GCG GTG TTT TCA GAA GTG GCT CGC ATC 700
Val Leu Leu Ala Val Pro Glu Ala Val Phe Ser Glu Val Ala Arg Ile
175 180 185

AGT AGC TTG GAT AAT AGC AGC TTC ACA GCA TGT ATC CCA TAC CCT CAA 748
Ser Ser Leu Asp Asn Ser Ser Phe Thr Ala Cys Ile Pro Tyr Pro Gln
190 195 200

ACA GAT GAA TTA CAT CCA AAG ATT CAT TCA GTG CTC ATT TTC TTG GTC 796
Thr Asp Glu Leu His Pro Lys Ile His Ser Val Leu Ile Phe Leu Val
205 210 215

TAT TTC CTC ATA CCA CTT GCT ATT ATT AGC ATT TAT TAT TAT CAT ATT 844
Tyr Phe Leu Ile Pro Leu Ala Ile Ile Ser Ile Tyr Tyr Tyr His Ile
220 225 230 235

GCA AAG ACC TTA ATT AAA AGC GCA CAC AAT CTT CCT GGA GAA TAC AAT 892
Ala Lys Thr Leu Ile Lys Ser Ala His Asn Leu Pro Gly Glu Tyr Asn
240 245 250

GAA CAT ACC AAA AAA CAG ATG GAA ACA CGG AAA CGC CTG GCT AAA ATT 940
Glu His Thr Lys Lys Gln Met Glu Thr Arg Lys Arg Leu Ala Lys Ile
255 260 265

GTG CTT GTC TTT GTG GGC TGT TTC ATC TTC TGT TGG TTT CCA AAC CAC 988
Val Leu Val Phe Val Gly Cys Phe Ile Phe Cys Trp Phe Pro Asn His
270 275 280

```
ATC CTT TAC ATG TAT CGG TCT TTC AAC TAT AAT GAG ATT GAT CCA TCT      1036
Ile Leu Tyr Met Tyr Arg Ser Phe Asn Tyr Asn Glu Ile Asp Pro Ser
    285                 290                 295

CTA GGC CAC ATG ATT GTC ACC TTA GTT GCC CGG GTT CTC AGT TTT GGC      1084
Leu Gly His Met Ile Val Thr Leu Val Ala Arg Val Leu Ser Phe Gly
300                 305                 310                 315

AAT TCT TGT GTC AAC CCA TTT GCT CTT TAC CTA CTC AGT GAA AGC TTC      1132
Asn Ser Cys Val Asn Pro Phe Ala Leu Tyr Leu Leu Ser Glu Ser Phe
                320                 325                 330

AGG AGG CAT TTC AAC AGC CAA CTC TGC TGT GGG AGG AAG TCC TAT CAA      1180
Arg Arg His Phe Asn Ser Gln Leu Cys Cys Gly Arg Lys Ser Tyr Gln
            335                 340                 345

GAG AGA GGA ACC AGC TAC CTA CTC AGC TCT TCA GCG GTG CGT ATG ACA      1228
Glu Arg Gly Thr Ser Tyr Leu Leu Ser Ser Ser Ala Val Arg Met Thr
        350                 355                 360

TCT CTG AAA AGC AAT GCT AAG AAC ATG GTG ACC AAT TCT GTT TTA CTA      1276
Ser Leu Lys Ser Asn Ala Lys Asn Met Val Thr Asn Ser Val Leu Leu
    365                 370                 375

AAT GGG CAC AGC ATG AAG CAG GAA ATG GCA ATG TGATTTTGGC CATTCAACTC    1329
Asn Gly His Ser Met Lys Gln Glu Met Ala Met
380                 385                 390

ACTACCTGGA GAGAACTTAG TAA                                           1352
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 390 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Pro Ser Lys Ser Leu Ser Asn Leu Ser Val Thr Thr Gly Ala Asn
  1               5                  10                  15

Glu Ser Gly Ser Val Pro Glu Gly Trp Glu Arg Asp Phe Leu Pro Ala
             20                  25                  30

Ser Asp Gly Thr Thr Thr Glu Leu Val Ile Arg Cys Val Ile Pro Ser
         35                  40                  45

Leu Tyr Leu Leu Ile Ile Thr Val Gly Leu Leu Gly Asn Ile Met Leu
     50                  55                  60

Val Lys Ile Phe Ile Thr Asn Ser Ala Met Arg Ser Val Pro Asn Ile
 65                  70                  75                  80
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Phe Ile Ser Asn Leu Ala Ala Gly Asp Leu Leu Leu Leu Leu Thr Cys
85 90 95

Val Pro Val Asp Ala Ser Arg Tyr Phe Phe Asp Glu Trp Met Phe Gly
100 105 110

Lys Val Gly Cys Lys Leu Ile Pro Val Ile Gln Leu Thr Ser Val Gly
115 120 125

Val Ser Val Phe Thr Leu Thr Ala Leu Ser Ala Asp Arg Tyr Arg Ala
130 135 140

Ile Val Asn Pro Met Asp Met Gln Thr Ser Gly Ala Leu Leu Arg Thr
145 150 155 160

Cys Val Lys Ala Met Gly Ile Trp Val Val Ser Val Leu Leu Ala Val
165 170 175

Pro Glu Ala Val Phe Ser Glu Val Ala Arg Ile Ser Ser Leu Asp Asn
180 185 190

Ser Ser Phe Thr Ala Cys Ile Pro Tyr Pro Gln Thr Asp Glu Leu His
195 200 205

Pro Lys Ile His Ser Val Leu Ile Phe Leu Val Tyr Phe Leu Ile Pro
210 215 220

Leu Ala Ile Ile Ser Ile Tyr Tyr Tyr His Ile Ala Lys Thr Leu Ile
225 230 235 240

Lys Ser Ala His Asn Leu Pro Gly Glu Tyr Asn Glu His Thr Lys Lys
245 250 255

Gln Met Glu Thr Arg Lys Arg Leu Ala Lys Ile Val Leu Val Phe Val
260 265 270

Gly Cys Phe Ile Phe Cys Trp Phe Pro Asn His Ile Leu Tyr Met Tyr
275 280 285

Arg Ser Phe Asn Tyr Asn Glu Ile Asp Pro Ser Leu Gly His Met Ile
290 295 300

Val Thr Leu Val Ala Arg Val Leu Ser Phe Gly Asn Ser Cys Val Asn
305 310 315 320

Pro Phe Ala Leu Tyr Leu Leu Ser Glu Ser Phe Arg Arg His Phe Asn
325 330 335

Ser Gln Leu Cys Cys Gly Arg Lys Ser Tyr Gln Glu Arg Gly Thr Ser
340 345 350

Tyr Leu Leu Ser Ser Ser Ala Val Arg Met Thr Ser Leu Lys Ser Asn
355 360 365

Ala Lys Asn Met Val Thr Asn Ser Val Leu Leu Asn Gly His Ser Met
370 375 380

Lys Gln Glu Met Ala Met
385 390

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1606 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 172..1371

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAAACACAGA ACTGAAGCAA AGGAGTATCT GGATGTCTTG GATTTTCTTC CCATTCTGTT 60

CTGTTCTGTT CTCCTAATAC CATCTCGTTA CTAGACGTAG GCATTGGACG TGACAATCAA 120

CTGCATTTGA ACTGAGAAGA AGAAATATTA AAGACACAGT CTTCAGAAGA A ATG GCT 177
Met Ala
1

CAA AGG CAG CCT CAC TCA CCT AAT CAG ACT TTA ATT TCA ATC ACA AAT 225
Gln Arg Gln Pro His Ser Pro Asn Gln Thr Leu Ile Ser Ile Thr Asn
5 10 15

GAC ACA GAA TCA TCA AGC TCT ATG GTT TCT AAC GAT AAC ACA AAT AAA 273
Asp Thr Glu Ser Ser Ser Ser Met Val Ser Asn Asp Asn Thr Asn Lys
20 25 30

GGA TGG AGC GGG GAC AAC TCT CCA GGA ATA GAA GCA TTG TGT GCC ATC 321
Gly Trp Ser Gly Asp Asn Ser Pro Gly Ile Glu Ala Leu Cys Ala Ile
35 40 45 50

TAT ATT ACT TAT GCT GTG ATC ATT TCA GTG GGC ATC CTT GGA AAT GCT 369
Tyr Ile Thr Tyr Ala Val Ile Ile Ser Val Gly Ile Leu Gly Asn Ala
55 60 65

ATT CTC ATC AAA GTC TTT TTC AAG ACC AAA TCC ATG CAA ACA GTT CCA 417
Ile Leu Ile Lys Val Phe Phe Lys Thr Lys Ser Met Gln Thr Val Pro
70 75 80

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AAT ATT TTC ATC ACC AGC CTG GCT TTT GGA GAT CTT TTA CTT CTG CTA      465
Asn Ile Phe Ile Thr Ser Leu Ala Phe Gly Asp Leu Leu Leu Leu Leu
          85                  90                  95

ACT TGT GTG CCA GTG GAT GCA ACT CAC TAC CTT GCA GAA GGA TGG CTG      513
Thr Cys Val Pro Val Asp Ala Thr His Tyr Leu Ala Glu Gly Trp Leu
      100                 105                 110

TTC GGA AGA ATT GGT TGT AAG GTG CTC TCT TTC ATC CGG CTC ACT TCT      561
Phe Gly Arg Ile Gly Cys Lys Val Leu Ser Phe Ile Arg Leu Thr Ser
115                 120                 125                 130

GTT GGT GTG TCA GTG TTC ACA TTA ACA ATT CTC AGC GCT GAC AGA TAC      609
Val Gly Val Ser Val Phe Thr Leu Thr Ile Leu Ser Ala Asp Arg Tyr
                135                 140                 145

AAG GCA GTT GTG AAG CCA CTT GAG CGA CAG CCC TCC AAT GCC ATC CTG      657
Lys Ala Val Val Lys Pro Leu Glu Arg Gln Pro Ser Asn Ala Ile Leu
            150                 155                 160

AAG ACT TGT GTA AAA GCT GGC TGC GTC TGG ATC GTG TCT ATG ATA TTT      705
Lys Thr Cys Val Lys Ala Gly Cys Val Trp Ile Val Ser Met Ile Phe
        165                 170                 175

GCT CTA CCT GAG GCT ATA TTT TCA AAT GTA TAC ACT TTT CGA GAT CCC      753
Ala Leu Pro Glu Ala Ile Phe Ser Asn Val Tyr Thr Phe Arg Asp Pro
    180                 185                 190

AAT AAA AAT ATG ACA TTT GAA TCA TGT ACC TCT TAT CCT GTC TCT AAG      801
Asn Lys Asn Met Thr Phe Glu Ser Cys Thr Ser Tyr Pro Val Ser Lys
195                 200                 205                 210

AAG CTC TTG CAA GAA ATA CAT TCT CTG CTG TGC TTC TTA GTG TTC TAC      849
Lys Leu Leu Gln Glu Ile His Ser Leu Leu Cys Phe Leu Val Phe Tyr
                215                 220                 225

ATT ATT CCA CTC TCT ATT ATC TCT GTC TAC TAT TCC TTG ATT GCT AGG      897
Ile Ile Pro Leu Ser Ile Ile Ser Val Tyr Tyr Ser Leu Ile Ala Arg
            230                 235                 240

ACC CTT TAC AAA AGC ACC CTG AAC ATA CCT ACT GAG GAA CAA AGC CAT      945
Thr Leu Tyr Lys Ser Thr Leu Asn Ile Pro Thr Glu Glu Gln Ser His
        245                 250                 255

GCC CGT AAG CAG ATT GAA TCC CGA AAG AGA ATT GCC AGA ACG GTA TTG      993
Ala Arg Lys Gln Ile Glu Ser Arg Lys Arg Ile Ala Arg Thr Val Leu
    260                 265                 270

GTG TTG GTG GCT CTG TTT GCC CTC TGC TGG TTG CCA AAT CAC CTC CTG     1041
Val Leu Val Ala Leu Phe Ala Leu Cys Trp Leu Pro Asn His Leu Leu
275                 280                 285                 290

TAC CTC TAC CAT TCA TTC ACT TCT CAA ACC TAT GTA GAC CCC TCT GCC     1089
Tyr Leu Tyr His Ser Phe Thr Ser Gln Thr Tyr Val Asp Pro Ser Ala
                295                 300                 305
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```
ATG CAT TTC ATT TTC ACC ATT TTC TCT CGG GTT TTG GCT TTC AGC AAT      1137
Met His Phe Ile Phe Thr Ile Phe Ser Arg Val Leu Ala Phe Ser Asn
            310                 315                 320

TCT TGC GTA AAC CCC TTT GCT CTC TAC TGG CTG AGC AAA AGC TTC CAG      1185
Ser Cys Val Asn Pro Phe Ala Leu Tyr Trp Leu Ser Lys Ser Phe Gln
        325                 330                 335

AAG CAT TTT AAA GCT CAG TTG TTC TGT TGC AAG GCG GAG CGG CCT GAG      1233
Lys His Phe Lys Ala Gln Leu Phe Cys Cys Lys Ala Glu Arg Pro Glu
    340                 345                 350

CCT CCT GTT GCT GAC ACC TCT CTT ACC ACC CTG GCT GTG ATG GGA ACG      1281
Pro Pro Val Ala Asp Thr Ser Leu Thr Thr Leu Ala Val Met Gly Thr
355                 360                 365                 370

GTC CCG GGC ACT GGG AGC ATA CAG ATG TCT GAA ATT AGT GTG ACC TCG      1329
Val Pro Gly Thr Gly Ser Ile Gln Met Ser Glu Ile Ser Val Thr Ser
                375                 380                 385

TTC ACT GGG TGT AGT GTG AAG CAG GCA GAG GAC AGA TTC TAGCTTTTCA      1378
Phe Thr Gly Cys Ser Val Lys Gln Ala Glu Asp Arg Phe
            390                 395                 400

AGGAAAAATG CTGCTTCTCC TCCCAGCGTG TGTATCCGAC TCTAAGCTGT GTGCAGGTGT    1438

ATGGTGTCCA GATTTTTGTT GTTTGAAAAG TGTGTTGAAA TCTTAGGAGT GAAGGATCCC    1498

TATAAGTAAG TAAAATACAA ACCATTACTT TCTTCAAAGT ACAAATAGTA ATGTCATCGG    1558

CTTCTAATAA ATGAGCCCAC TAGTGCAGAA AGACAGTTTA TATATGCC                 1606
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 399 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Ala Gln Arg Gln Pro His Ser Pro Asn Gln Thr Leu Ile Ser Ile
 1               5                  10                  15

Thr Asn Asp Thr Glu Ser Ser Ser Ser Met Val Ser Asn Asp Asn Thr
            20                  25                  30

Asn Lys Gly Trp Ser Gly Asp Asn Ser Pro Gly Ile Glu Ala Leu Cys
        35                  40                  45

Ala Ile Tyr Ile Thr Tyr Ala Val Ile Ile Ser Val Gly Ile Leu Gly
    50                  55                  60
```

Asn Ala Ile Leu Ile Lys Val Phe Phe Lys Thr Lys Ser Met Gln Thr
65 70 75 80

Val Pro Asn Ile Phe Ile Thr Ser Leu Ala Phe Gly Asp Leu Leu Leu
85 90 95

Leu Leu Thr Cys Val Pro Val Asp Ala Thr His Tyr Leu Ala Glu Gly
100 105 110

Trp Leu Phe Gly Arg Ile Gly Cys Lys Val Leu Ser Phe Ile Arg Leu
115 120 125

Thr Ser Val Gly Val Ser Val Phe Thr Leu Thr Ile Leu Ser Ala Asp
130 135 140

Arg Tyr Lys Ala Val Val Lys Pro Leu Glu Arg Gln Pro Ser Asn Ala
145 150 155 160

Ile Leu Lys Thr Cys Val Lys Ala Gly Cys Val Trp Ile Val Ser Met
165 170 175

Ile Phe Ala Leu Pro Glu Ala Ile Phe Ser Asn Val Tyr Thr Phe Arg
180 185 190

Asp Pro Asn Lys Asn Met Thr Phe Glu Ser Cys Thr Ser Tyr Pro Val
195 200 205

Ser Lys Lys Leu Leu Gln Glu Ile His Ser Leu Leu Cys Phe Leu Val
210 215 220

Phe Tyr Ile Ile Pro Leu Ser Ile Ile Ser Val Tyr Tyr Ser Leu Ile
225 230 235 240

Ala Arg Thr Leu Tyr Lys Ser Thr Leu Asn Ile Pro Thr Glu Glu Gln
245 250 255

Ser His Ala Arg Lys Gln Ile Glu Ser Arg Lys Arg Ile Ala Arg Thr
260 265 270

Val Leu Val Leu Val Ala Leu Phe Ala Leu Cys Trp Leu Pro Asn His
275 280 285

Leu Leu Tyr Leu Tyr His Ser Phe Thr Ser Gln Thr Tyr Val Asp Pro
290 295 300

Ser Ala Met His Phe Ile Phe Thr Ile Phe Ser Arg Val Leu Ala Phe
305 310 315 320

Ser Asn Ser Cys Val Asn Pro Phe Ala Leu Tyr Trp Leu Ser Lys Ser
325 330 335

Phe Gln Lys His Phe Lys Ala Gln Leu Phe Cys Cys Lys Ala Glu Arg
340 345 350

Pro Glu Pro Pro Val Ala Asp Thr Ser Leu Thr Thr Leu Ala Val Met
355 360 365

Gly Thr Val Pro Gly Thr Gly Ser Ile Gln Met Ser Glu Ile Ser Val
370 375 380

Thr Ser Phe Thr Gly Cys Ser Val Lys Gln Ala Glu Asp Arg Phe
385 390 395

WHAT IS CLAIMED IS:

1. A DNA segment coding for a polypeptide having an amino acid sequence corresponding to a human gastrin releasing peptide-receptor, or a unique portion thereof.

2. The DNA segment according to Claim 1, wherein said DNA segment has the sequence shown in SEQ ID NO: 7, allelic or species variation thereof, or a unique portion thereof.

3. The DNA segment according to Claim 1, wherein said DNA segment encodes the amino acid sequence set forth in SEQ ID NO: 8, allelic or species variation thereof, or a unique portion thereof.

4. A polypeptide free of proteins with which it is naturally associated and having an amino acid sequence corresponding to a human gastrin releasing peptide-receptor, or a unique portion thereof.

5. A polypeptide bound to a solid support and having an amino acid sequence corresponding to a human gastrin releasing peptide-receptor, or a unique portion thereof.

6. The polypeptide according to Claim 4 or 5, wherein said polypeptide has the amino acid sequence set forth in SEQ ID NO: 8, allelic or species variation thereof, or a unique portion thereof.

7. A recombinant DNA molecule comprising a vector and the DNA segment according to Claim 1.

8. A cell that contains the recombinant DNA molecule according to Claim 7.

9. A method of producing a polypeptide having an amino acid sequence corresponding to human gastrin releasing peptide-receptor comprising culturing the cell according to Claim 8

under conditions such that said DNA segment is expressed and said polypeptide thereby produced, and isolating said polypeptide.

10. An antibody having binding affinity to a recombinant human gastrin releasing peptide-receptor, or unique portions thereof.

11. The antibody according to Claim 10, wherein said receptor has the amino acid sequence set forth in SEQ ID NO: 8, allelic or species variation thereof, or a unique portion thereof.

12. A DNA segment coding for a polypeptide having an amino acid sequence corresponding to a neuromedin-B-preferring bombesin receptor, or a unique portion thereof.

13. The DNA segment according to Claim 12, wherein said DNA segment has the sequence shown in SEQ ID No:5, allelic or species variation thereof, or a unique portion thereof.

14. The DNA segment according to Claim 12, wherein said DNA segment encodes the amino acid sequence set forth in SEQ ID NO: 6, allelic or species variation thereof, or a unique portion thereof.

15. A polypeptide free of proteins with which it is naturally associated and having an amino acid sequence corresponding to a neuromedin-B-preferring bombesin receptor, or a unique portion thereof.

16. A polypeptide bound to a solid support and having an amino acid sequence corresponding to a neuromedin-B-preferring bombesin receptor, or a unique portion thereof.

17. The polypeptide according to Claim 15 or 16, wherein said polypeptide has the amino acid sequence set forth in SEQ ID NO: 6, allelic or species variation thereof, or a unique portion thereof.

18. A recombinant DNA molecule comprising a vector and the DNA segment according to Claim 12.

19. A cell that contains the recombinant DNA molecule according to Claim 18.

20. A method of producing a polypeptide having an amino acid sequence corresponding to neuromedin-B-preferring bombesin receptor comprising culturing the cell according to Claim 19 under conditions such that said DNA segment is expressed and said polypeptide thereby produced, and isolating said polypeptide.

21. An antibody having binding affinity to a recombinant neuromedin-B-preferring bombesin receptor, or unique portions thereof.

22. The antibody according to Claim 21, wherein said receptor has the amino acid sequence set forth in SEQ ID NO: 6, allelic or species variation thereof, or a unique portion thereof.

23. A recombinant or substantially pure nucleic acid comprising a sequence exhibiting substantial homology to a nucleotide sequence encoding a receptor, or a fragment thereof, for a bombesin-like peptide.

24. A nucleic acid of Claim 23 further comprising sequence encoding a second polypeptide, or fragment thereof.

25. A vector, cell, or organism comprising a nucleic acid of Claim 23.

26. A recombinant or substantially pure polypeptide comprising a region exhibiting substantial identity to an amino acid fragment of a receptor for a bombesin-like peptide.

27. A polypeptide of Claim 26 comprising a fragment of a second polypeptide.

28. A subcellular structure, cell, or organism comprising a protein of Claim 26.

29. A method of producing a receptor, or fragment thereof, for a bombesin-like peptide comprising expressing a nucleic acid of Claim 23.

30. A method of screening for a compound having binding affinity to a receptor for a bombesin-like peptide comprising the steps of:

a) producing said receptor by a method of Claim 29, and

b) assaying for the binding of said compound to said receptor.

31. An antibody having binding affinity for a receptor for a bombesin-like peptide or fragment thereof.

32. A method of simultaneously modulating a biological activity of a plurality of subtypes of receptors for bombesin-like peptides, comprising contacting said receptors with a compound which modulates said activity upon contacting said receptors.

33. An antibody exhibiting specificity of binding to at least one receptor for a bombesin-like peptide selected from the group consisting of:

a) a mouse R1BP, or fragment thereof;

b) a human R1BP, or fragment thereof;

c) a rat R2BP, or fragment thereof;

d) a human R2BP, or fragment thereof; and

e) a human R3BP, or fragment thereof.

34. A method of modulating biological activity of a receptor for a bombesin-like peptide comprising contacting said receptor with a composition selected from the group consisting of:

a) an antibody which binds to said receptor;

b) a known agonist or antagonist to a receptor for a non-GRP bombesin-like peptide; and

c) a ligand binding fragment from a receptor for a bombesin-like peptide.

35. A method of treating a host having cancer or exhibiting abnormal expression of a receptor for a bombesin-like peptide, comprising administering to said host a therapeutically effective amount of a composition comprising:

a) an antibody which binds to a receptor for a bombesin-like peptide;

b) an agonist or antagonist to a receptor for a non-GRP bombesin-like peptide; or

c) a ligand binding receptor, or fragment thereof, for a bombesin-like peptide.

36. A method of diagnosing for cancer in a host organism, comprising the steps of:

a) contacting a sample from said host with a specific binding reagent to:

i) a gene encoding a receptor for a bombesin-like peptide; or

ii) a receptor for a bombesin-like peptide; and

b) measuring the level of binding of said reagent to said sample.

37. A method of evaluating binding affinity of a test compound to a receptor for a bombesin-like peptide, said method comprising the steps of:

a) contacting a sample containing said receptor with

i) a labeled compound having a known affinity for said receptor; and

ii) said test compound; and

b) measuring the level of bound labeled compound, said amount being inversely proportional to the amount of test compound which bound to said receptor.

38. A kit for determining the amount of a receptor for a bombesin-like peptide in a sample, comprising a compartment with a labeled compound having a known binding affinity for said receptor.

39. A kit for assaying antibody against a receptor for a bombesin-like peptide in a sample, comprising compartments having a said receptor and an antibody detection means.

40. A compound known to modulate activity of a receptor for a bombesin-like peptide, selected by a method of:

a) contacting said compound with isolated or recombinant receptor, or fragment thereof, for a bombesin-like peptide; and

b) evaluating the effect on biological activity by said contacting.

41. Isolated DNA encoding the gastrin releasing peptide receptor or fragment thereof encoding a biologically active gastrin releasing peptide receptor polypeptide.

42. Isolated DNA which encodes a biologically active protein having gastrin releasing peptide receptor activity and which is capable of hybridizing with the DNA of SEQ ID NO: 1.

43. The DNA of Claim 42 wherein said protein has the amino acid sequence of SEQ ID NO: 2.

44. Isolated DNA encoding proteins which are homologous to the gastrin releasing peptide receptor, and said DNA being isolated using gastrin releasing peptide receptor cDNA as a probe.

45. The DNA sequence according to Claims 41, 42, or 44 characterized in that it further comprises the respective regulatory sequences in the 5' and 3' flanks.

46. A DNA sequence hybridizing to a DNA sequence according to Claims 41, 42, or 44 and containing mutations selected from the group consisting of nucleotide substitutions, nucleotide deletions, nucleotide insertions and inversions of nucleotide stretches and coding for a protein having gastrin releasing peptide receptor activity.

47. A recombinant DNA molecule characterized in that it comprises a DNA sequence according to Claims 41, 42, or 44.

48. A recombinant DNA molecule characterized in that it comprises a DNA sequence according to Claims 41, 42, or 44 that is operably linked to a genetic control element.

49. The recombinant DNA molecule of Claim 48, characterized in that said control element is selected from the group consisting of procaryotic promoter systems and eucaryotic expression control
systems.

50. The recombinant molecule of Claim 47 wherein said molecule is an expression vector for expressing eucaryotic cDNA coding for the gastrin releasing peptide receptor in a procaryotic or eucaryotic host, said vector being compatible with said host and wherein the eucaryotic cDNA coding for the gastrin releasing peptide receptor is inserted into said vector such that growth of the host containing said vector expresses said cDNA.

51. A host characterized in that the recombinant DNA molecule according to Claim 47 has been introduced into said host, and which expresses the protein encoded by said DNA.

52. The host of Claim 51 which is selected from the group consisting of: procaryotes including gram negative and gram positive organisms including E. coli; lower eucaryotes including yeasts; and higher eucaryotes including animal cells and mammalian cells including human.

53. A recombinant protein which is encoded by a DNA sequence according to Claim 47 and which is substantially free of protein or cellular contaminants, other than those derived from the recombinant host.

54. A pharmaceutical composition comprising the recombinant protein of Claim 53 and a conventional pharmaceutically acceptable carrier and/or diluent.

55. A vector comprising DNA encoding the gastrin releasing peptide receptor or a fragment thereof encoding a biologically active gastrin releasing peptide receptor polypeptide.

56. The vector of Claim 55 wherein said DNA is under the control of a viral promoter.

57. The vector of Claim 55 which further comprises DNA encoding a selection marker.

58. The vector of Claim 55 wherein said DNA encodes a predetermined, site-specific mutant gastrin releasing peptide receptor which has greater than about 50% amino acid homology with the gastrin releasing peptide receptor of SEQ ID NO: 2 and which exhibits biological activity in common with the gastrin releasing peptide receptor of SEQ ID NO: 2.

59. A cell from a multicellular organism transformed with the vector of Claim 55.

60. The cell of Claim 59 which is a mammalian cell.

61. A method comprising culturing the cell of Claim 59 in a nutrient medium, permitting the receptor to accumulate in the culture and recovering the receptor from the culture.

62. The method of Claim 61 wherein the receptor is recovered from the culture medium.

63. Antibodies having binding affinity to the recombinant gastrin releasing peptide receptor, or fragments thereof.

64. The antibodies of Claim 63 which are raised against the gastrin releasing peptide receptor, or fragments thereof.

65. The antibodies of Claims 63 or 64 wherein said receptor has the amino acid sequence of SEQ ID NO: 2.

66. The antibodies of Claim 65 wherein said fragments are selected from the group consisting of the following partial amino acid sequences: residues 1-39, inclusive; residues 64-77, inclusive; residues 98-115, inclusive; residues 138-157, inclusive; residues 176-209, inclusive; residues 236-266, inclusive: residues 288-300, inclusive; and residues 330-385, inclusive.

67. The antibodies of Claim 63 which are non-neutralizing antibodies.

68. The antibodies of Claim 63 which are neutralizing antibodies.

69. The antibodies of Claim 63 which are conjugated to toxins.

70. The antibodies of Claim 63 which are conjugated to radionuclides.

71. A kit for determining the concentration of gastrin releasing peptide receptor in a sample comprising a labeled

compound having known binding affinity for the gastrin releasing peptide receptor, recombinant gastrin releasing peptide receptor, and a means for separating bound from free labeled compound.

72. The kit of Claim 71 wherein said means for separating is a solid phase for immobilizing the gastrin releasing peptide receptor.

73. The kit of Claim 71 wherein said labeled compound is a ligand.

74. The kit of Claim 73 wherein said ligand is gastrin releasing peptide.

75. The kit of Claim 71 wherein said labeled ligand is an antibody.

76. The kit of Claim 72 wherein said solid phase contains a capture molecule.

77. The kit of Claim 76 wherein said capture molecule is an antibody to the gastrin releasing peptide receptor.

78. A kit for determining the binding affinity of a test compound to the gastrin releasing peptide receptor comprising a test compound, a labeled compound having known binding affinity for the gastrin releasing peptide receptor, recombinant gastrin releasing peptide receptor and a means for separating bound from free labeled compound.

79. The kit of Claim 78 wherein said means for separating is a solid phase for immobilizing the solubilized gastrin releasing peptide receptor.

80. The kit of Claim 78 wherein said labeled compound is a ligand.

81. The kit of Claim 80 wherein said ligand is gastrin releasing peptide.

82. The kit of Claim 78 wherein said labeled ligand is an antibody.

83. The kit of Claim 79 wherein said solid phase contains a capture molecule.

84. The kit of Claim 83 wherein said capture molecule is an antibody to the gastrin releasing peptide receptor.

85. A method of treating patients having a disease or disorder associated with abnormal expression or abnormal triggering of the gastrin releasing peptide receptor comprising administering antibodies having binding affinity to the recombinant gastrin releasing peptide receptor.

86. A method of treating patients having a disease or disorder associated with abnormal expression or abnormal triggering of the gastrin releasing peptide receptor comprising administering recombinant gastrin releasing peptide receptor, or fragments thereof.

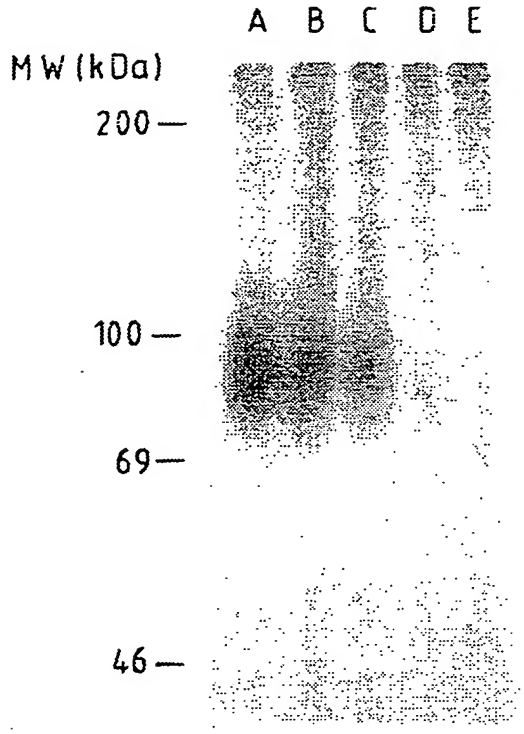

FIG. 5.

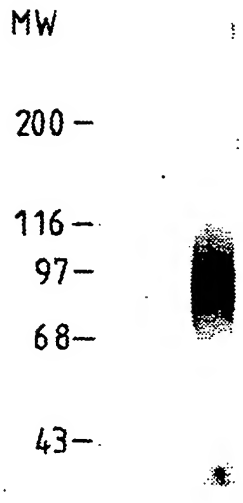

FIG. 6.

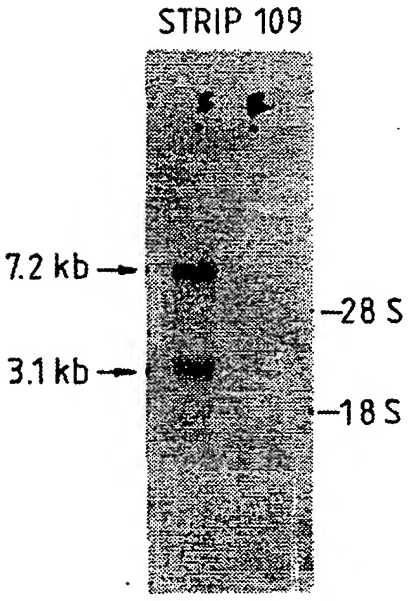

FIG. 9.

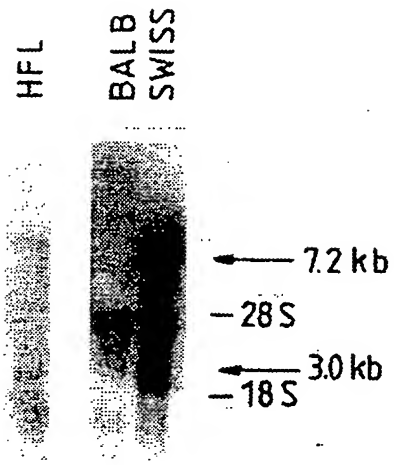

FIG. 10.

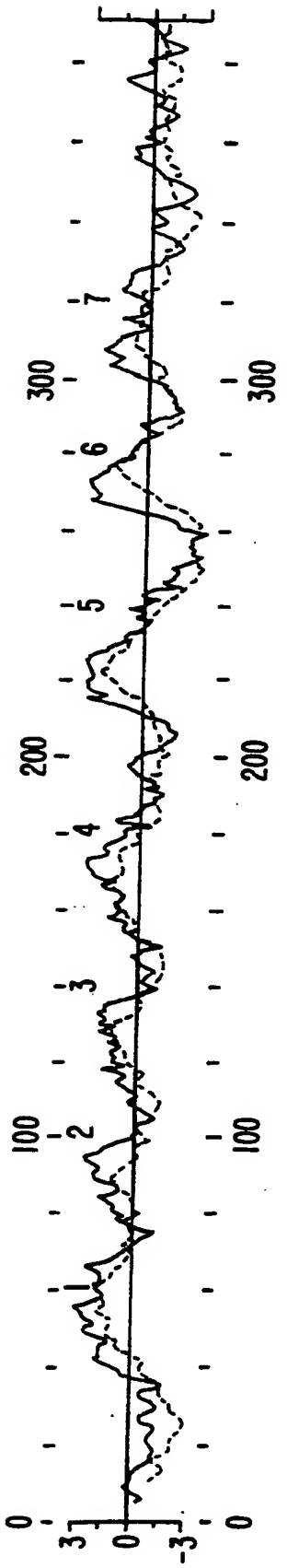
0
100
200
300
0
100
200
300
1
2
3
4
5
6
7
3
0
-3

FIG. 12.

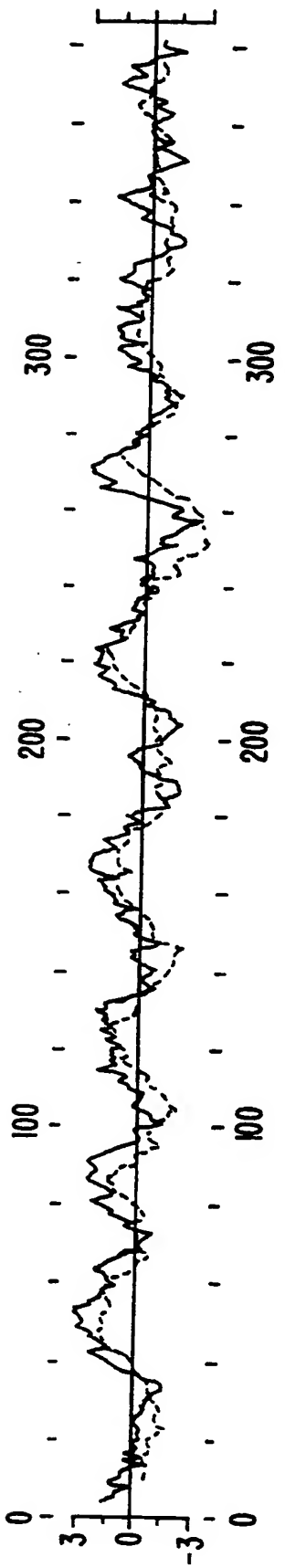
0
100
200
300
0
100
200
300
3
0
-3

FIG. 13.

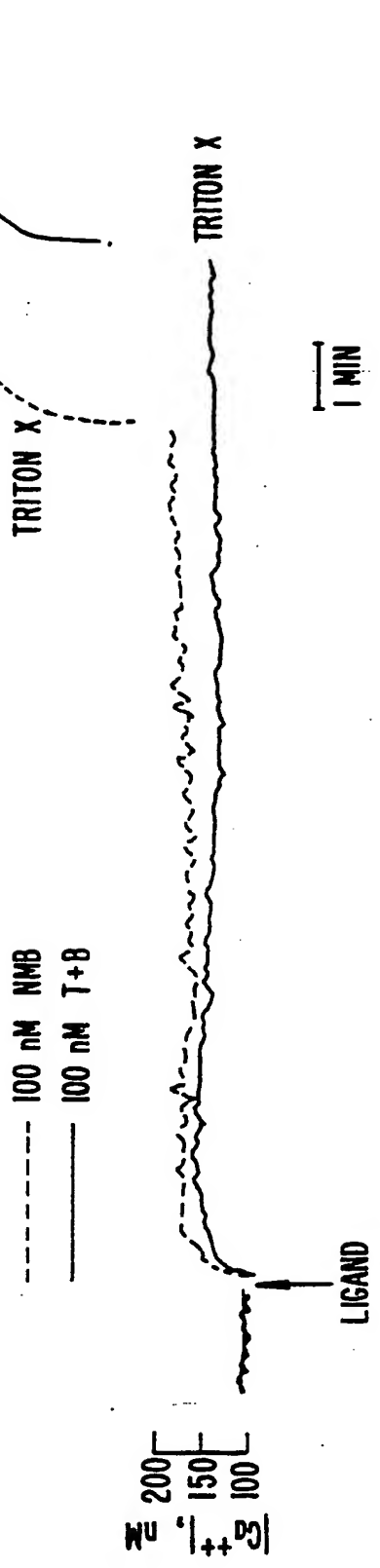
100 nM NMB
100 nM T+8
TRITON X
TRITON X
1 MIN
LIGAND
200
150
100
nM
$[Ca^{++}]_i$

FIG. 14.

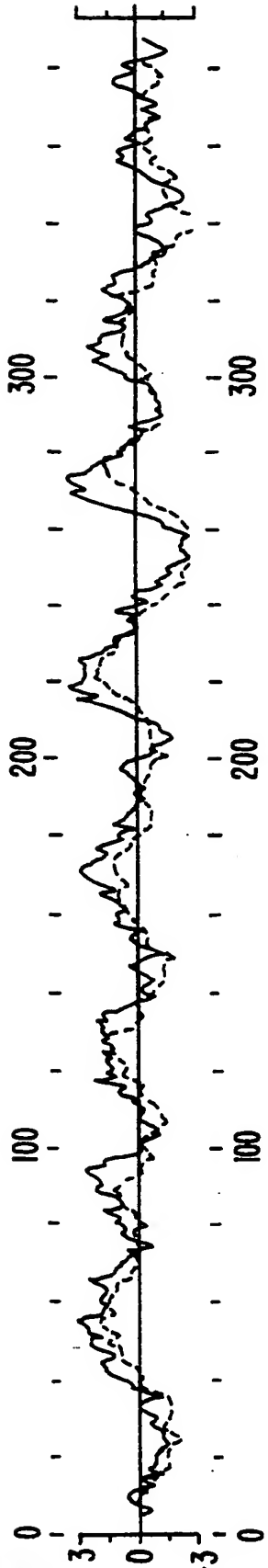
300
200
100
0
3
0
-3

FIG. 17.

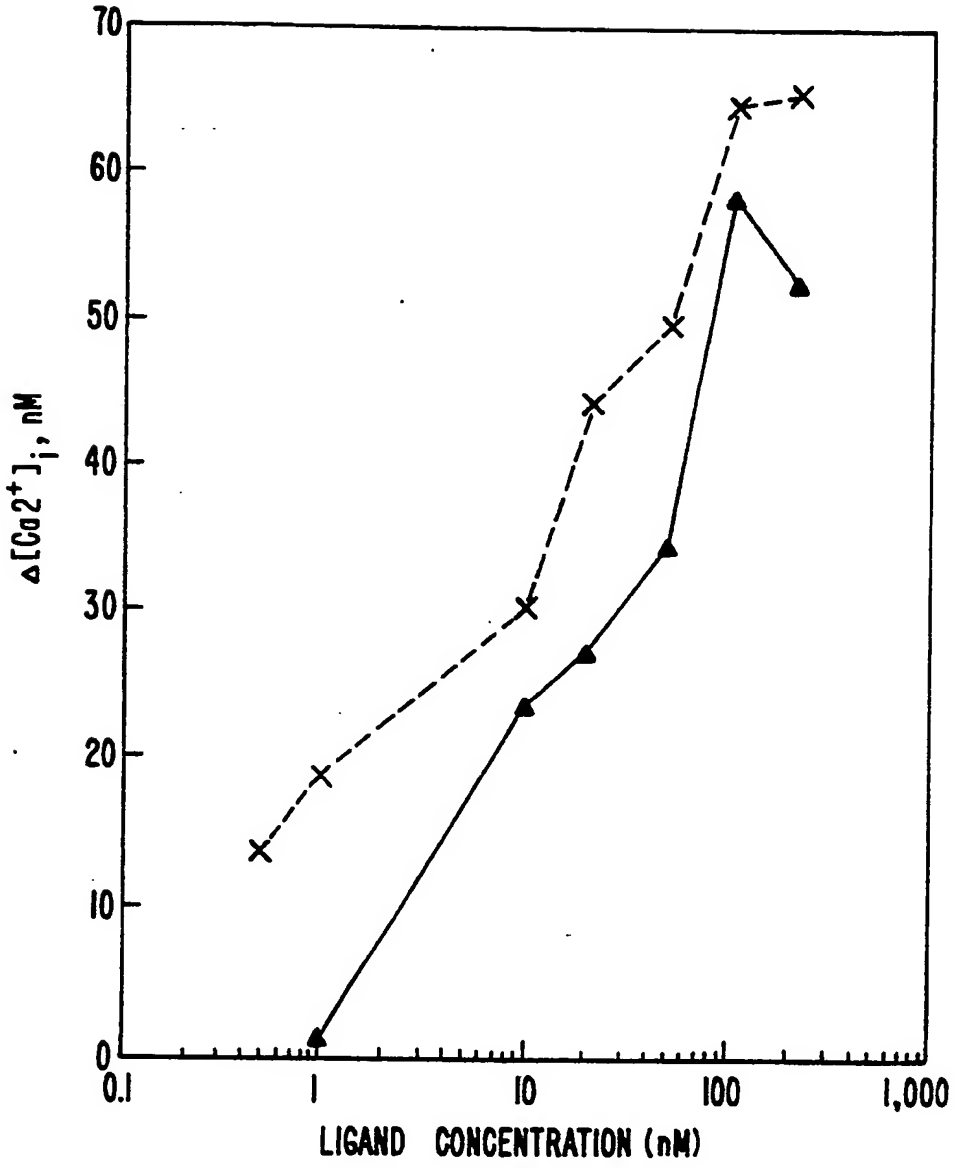
70
60
50
40
30
20
10
0
0.1
1
10
100
1,000
Δ[Ca2+]i, nM
LIGAND CONCENTRATION (nM)

FIG. 15.

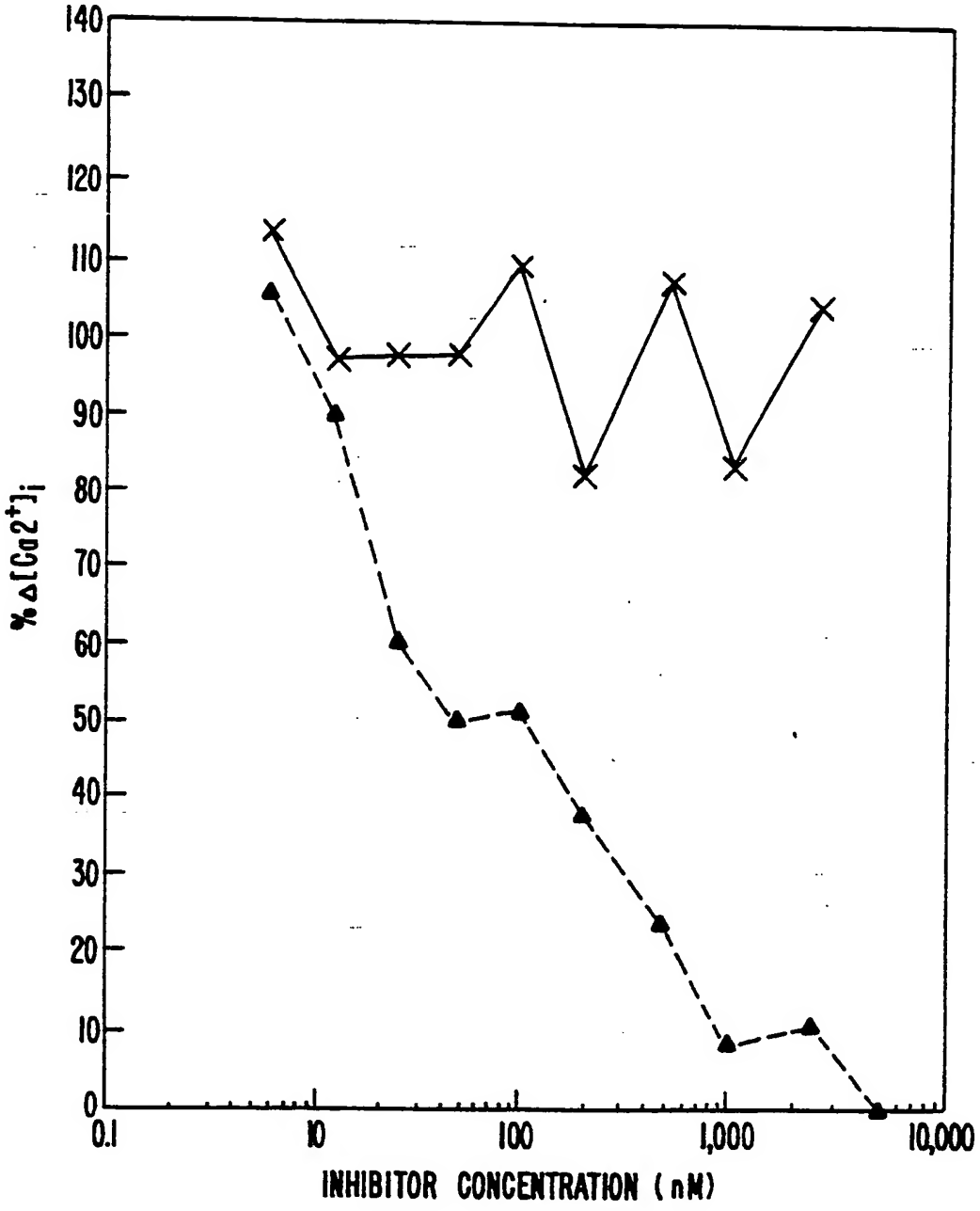
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
% Δ[Ca2+]i
0.1
10
100
1,000
10,000
INHIBITOR CONCENTRATION (nM)

FIG. 16.

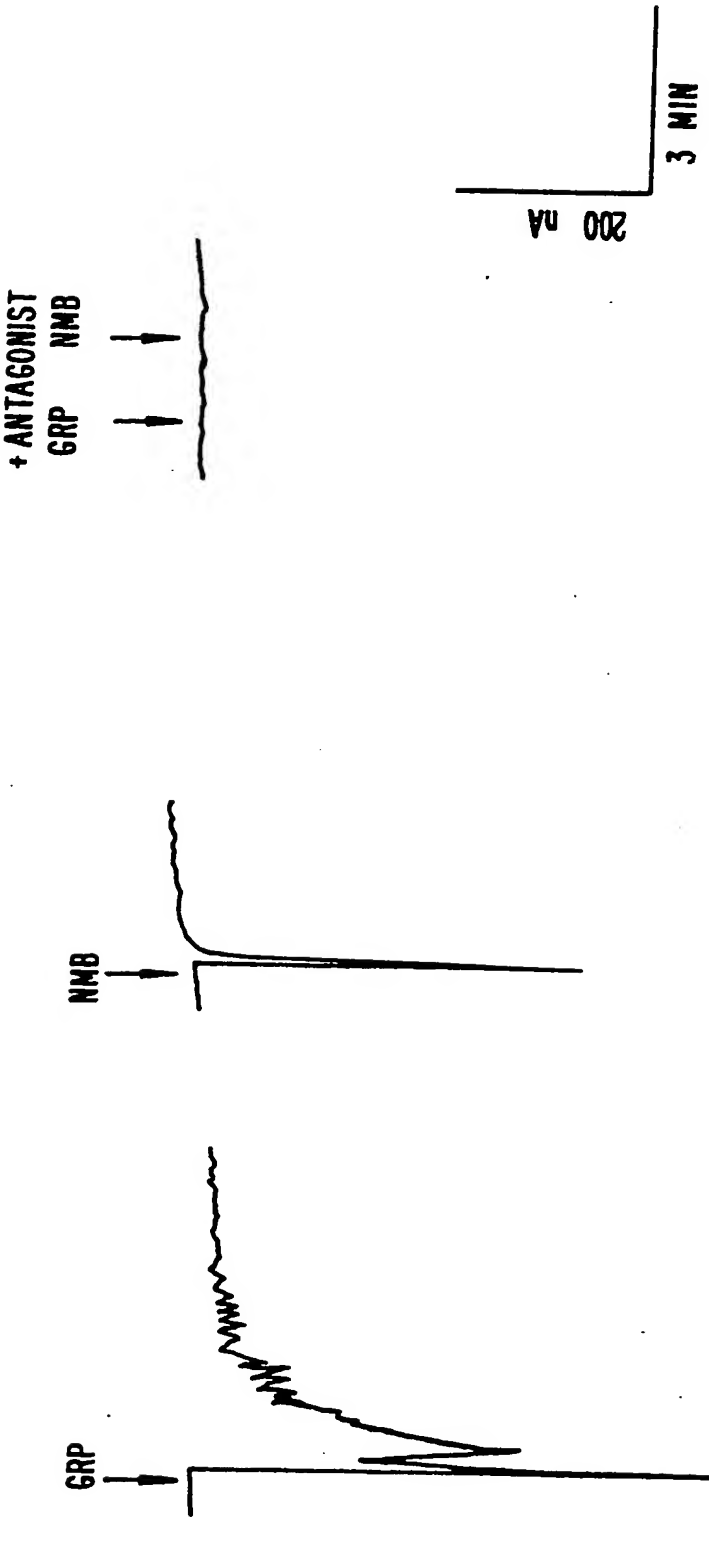

FIG. 18A.

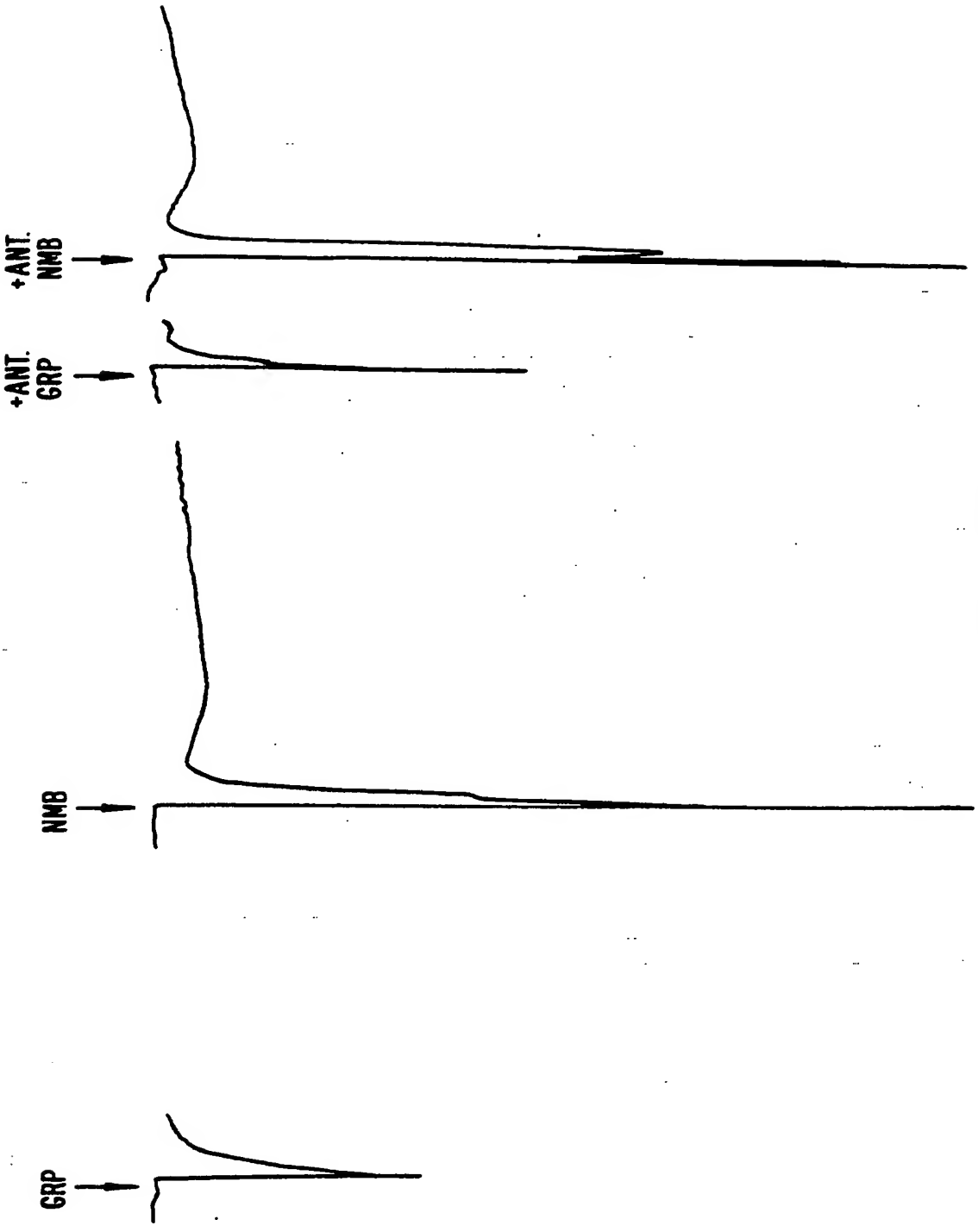

FIG. 18B.

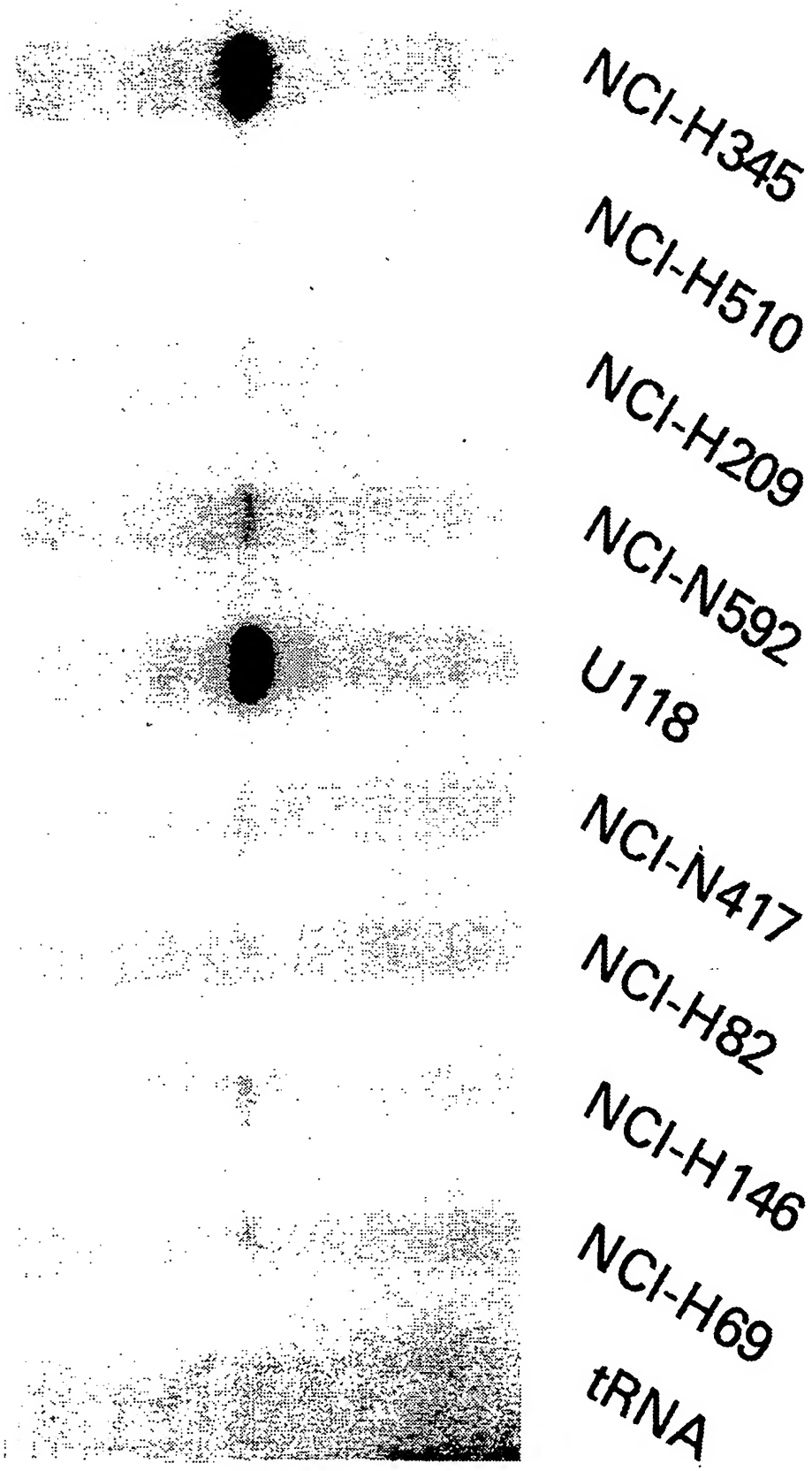
NCI-H345
NCI-H510
NCI-H209
NCI-N592
U118
NCI-N417
NCI-H82
NCI-H146
NCI-H69
tRNA

FIG. 19A.

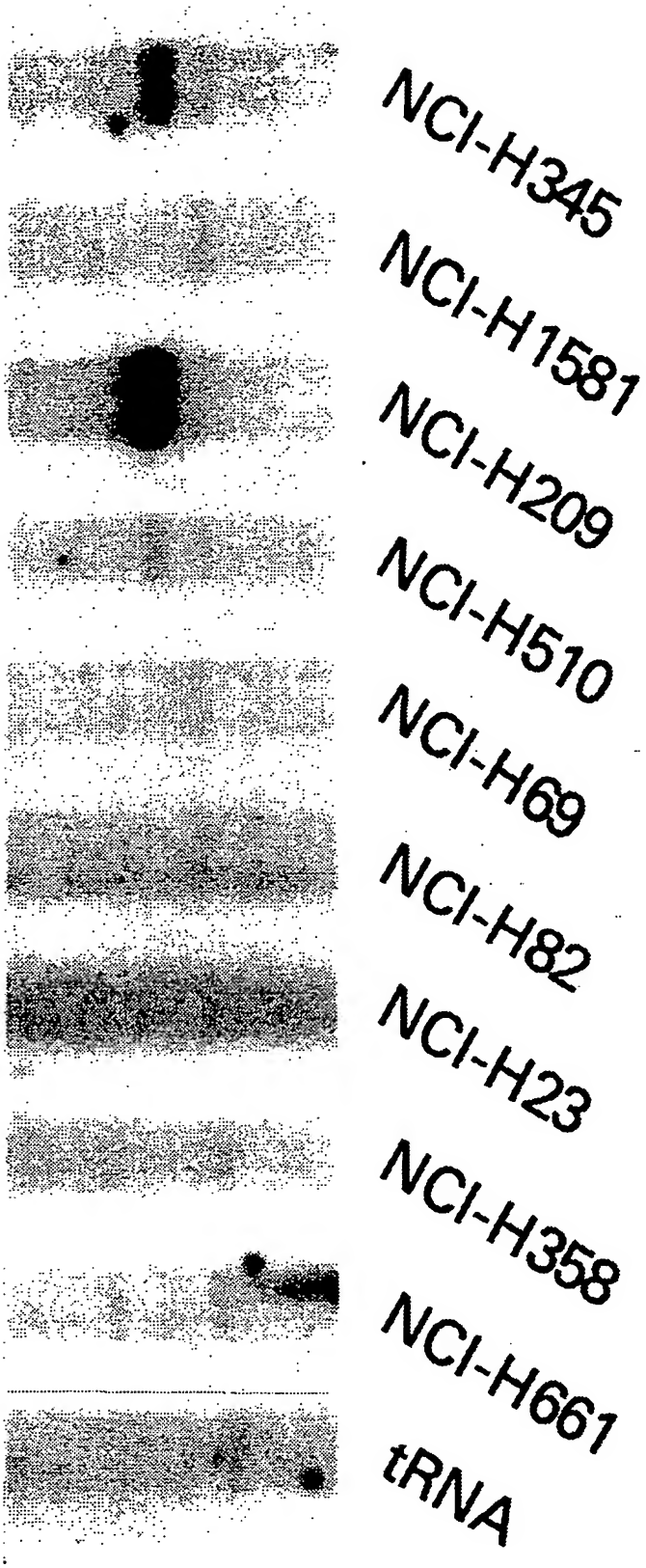
NCI-H345
NCI-H1581
NCI-H209
NCI-H510
NCI-H69
NCI-H82
NCI-H23
NCI-H358
NCI-H661
tRNA

FIG. 19B.

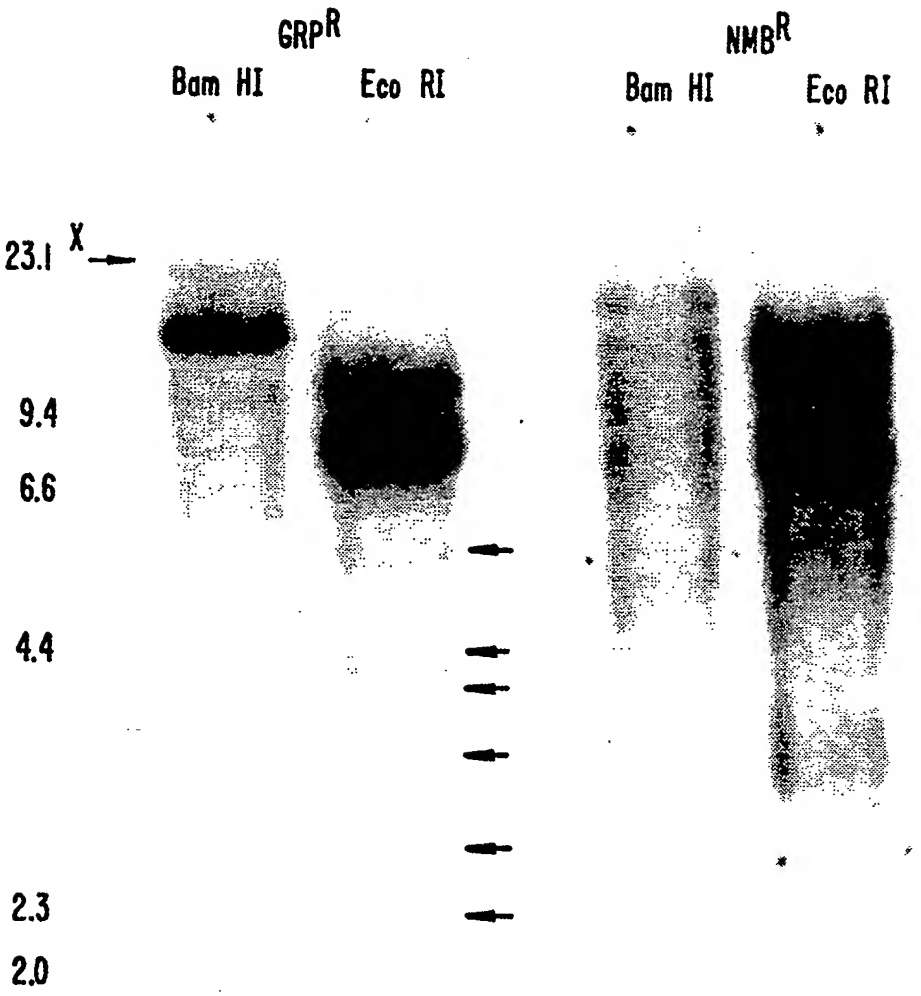

FIG. 20.

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